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SOUTHWEST TEXAS Q FEVER STUDIES*

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Since the first naturally acquired outbreak of *Coxiella burnetii* infections in the United States occurred in Amarillo, Texas (Irons et al., 1946) and (Topping et al., 1947), the disease has been revealed to be widespread in Texas by the occurrence of additional human cases, identification of the organism in raw milk samples from several dairies, and the demonstration of complement-fixing antibodies in sera from cattle, sheep and goats (Irons et al., 1949).

Although arthropods were found to have no discernible connection with the Amarillo Q fever cases, the fact that various species of ticks have been found naturally infected with the organism (Parker et al., 1949) has indicated the need for intensive studies relative to the role, if any, that arthropods play in the maintenance and spread of this disease.

The "W.C." ranch, encompassing approximately five thousand acres, the "W." dairy and adjacent areas in Zavala County, where Q fever was known to exist as a result of the occurrence of a human case and serological evidence in sheep and goats, were selected for these studies. Starting March 1, 1950, regular, total samples of ectoparasites were collected from wildlife and domestic animals on the ranch and immediate vicinity.

Sherman live traps were utilized in taking rodents, and Berlese funnels were of value in separating arthropods from rodent nesting material. Predatory animals were shot, or trapped in steel traps. Domestic animals were examined for ectoparasites in the field. Blood samples were obtained whenever possible.

The following species of ectoparasites were taken from March 1, 1950 through February 28, 1951, and suitably pooled for *C. burnetii* tests.

ORDER MARSUPIALIA

FAMILY DIDELPHIDAE

Opossum, *Didelphis virginiana*—5 examined.

Pulex irritans—43
Echidnophaga gallinacea—21
Bdellonyssus bacoti—1
Dermacentor variabilis—3

ORDER INSECTIVORA

FAMILY SORICIDAE

Shrew, *Notiosorex crawfordi*—1 examined.

Haemolaelaps glasgowi—1
Ixodes woodi—2

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ORDER RODENTIA

FAMILY HETEROMYIDAE

Kangaroo rat, *Dipodomys ordi*—11 examined.

No ectoparasites

Pocket mouse, *Perognathus hispidus*—43 examined.

Thrassis fotus—6
Orchopeas leucopus—2
Hoplopsyllus affinis—1
Androlaelaps grandiculatus—43
Bdellonyssus bacoti—7
Haemolaelaps glasgowi—1
Ornithodoros talaje—37

Pocket mouse, *Perognathus merriami*—6 examined.

Androlaelaps grandiculatus—9

FAMILY SCIURIDAE

Mexican ground squirrel, *Citellus mexicanus*—74 examined.

Thrassis fotus—243
Orchopeas sexdentatus—7
Hoplopsyllus affinis—1
Bdellonyssus bacoti—6
Haemolaelaps glasgowi—2
Amblyomma americanum—1
Haemaphysalis leporis-palustris—1
Ixodes woodi—1
Ornithodoros talaje—11
Enderleinellus suturalis—2

FAMILY CRICETIDAE

Deer mouse, *Peromyscus leucopus*—51 examined.

Orchopeas leucopus—52
Thrassis fotus—4
Hoplopsyllus affinis—1
Haemolaelaps glasgowi—1
Androlaelaps grandiculatus—4
Dermacentor variabilis—8
Ixodes woodi—2

Grasshopper mouse, *Onychomys leucogaster*—6 examined.

Eubrachylaelaps crowei—4
Haemolaelaps glasgowi—5
Bdellonyssus bacoti—11

Pack rat, *Neotoma micropus*—894 examined.

Orchopeas sexdentatus—2,376
Hoplopsyllus affinis—24
Pulex irritans—1
Orchopeas leucopus—18
Xenopsylla cheopis—1
Echidnophaga gallinacea—23
Bdellonyssus bacoti—82
Haemolaelaps glasgowi—16
Ixodes woodi—433
Ornithodoros talaje—335
Dermacentor variabilis—8
Neohaematopinus neotomae—23

Cotton rat, *Sigmodon hispidus*—606 examined.

Orchopeas leucopus—590
Orchopeas sexdentatus—3
Hoplopsyllus affinis—2
Bdellonyssus bacoti—536
Haemolaelaps glasgowi—241
Neoichoronyssus dentipes—11
Androlaelaps grandiculatus—1
Dermacentor variabilis—200
Ixodes woodi—8
Ornithodoros talaje—4
Hoplopleura hirsuta—551

FAMILY MURIDAE

Roof rat, *Rattus rattus*—13 examined.

Xenopsylla cheopis—1
Leptopsylla segnis—1
Orchopeas leucopus—18
Echidnophaga gallinacea—1
Bdellonyssus bacoti—1
Dermacentor variabilis—2

ORDER LAGOMORPHA

FAMILY LEPORIDAE

Jackrabbit, *Lepus californicus*—14 examined.

Hoplopsyllus affinis—15
Haemaphysalis leporis-palustris—77
Rhipicephalus sanguineus—27

Cottontail, *Sylvilagus auduboni*—42 examined.

Hoplopsyllus affinis—511
Haemaphysalis leporis-palustris—388
Amblyomma inornatum—2
Hoplopleura hirsuta—2

ORDER CARNIVORA

FAMILY MUSTELIDAE

Skunk, *Mephitis mephitis*—6 examined.

Pulex irritans—8
Juxtapulex porcinus—1
Amblyomma inornatum—3
Dermacentor variabilis—6

Badger, *Taxidea taxus*—3 examined.

Pulex irritans—5
Dermacentor variabilis—25
Ixodes kingi—3

FAMILY PROCYONIDAE

Raccoon, *Procyon lotor*—2 examined.

Pulex irritans—1
Dermacentor variabilis—39

FAMILY CANIDAE

Coyote, *Canis latrans*—37 examined.

Pulex irritans—57
Juxtapulex porcinus—20
Dermacentor variabilis—210
Amblyomma americanum—1
Heterodoxus spiniger—23

Fox, *Vulpes macrotis*—2 examined.

Pulex irritans—3
Dermacentor variabilis—12

Domestic dog—176 examined.

Pulex irritans—210
Echidnophaga gallinacea—47
Ctenocephalides felis—13
Juxtapulex porcinus—3
Rhipicephalus sanguineus—627
Dermacentor variabilis—52
Amblyomma americanum—8
Otobius megnini—3
Bovicola caprae—1

FAMILY FELIDAE

Bobcat, *Lynx rufus*—11 examined.

Juxtapulex porcinus—131
Hoplopsyllus affinis—4
Echidnophaga gallinacea—1
Dermacentor variabilis—113
Haemaphysalis leporis-palustris—1

Domestic cat—3 examined.

Dermacentor variabilis—12

ORDER ARTIODACTYLA

FAMILY TAYASSUIDAE

Javelina, *Tayassu angulatus*—6 examined.

Juxtapulex porcinus—22
Dermacentor variabilis—15

FAMILY CERVIDAE

Deer, *Odocoileus virginianus*—5 examined.

Ixodes scapularis—2
Amblyomma americanum—2
Dermacentor albipictus—3

Domestic goat—23 examined.

Amblyomma americanum—6
Haemaphysalis leporis-palustris—15
Linognathus africanus—11
Ixodes tovari—2

FAMILY BOVIDAE

Cow—215 examined.

Otobius megnini—553
Amblyomma americanum—23
Dermacentor variabilis—2
Haemaphysalis leporis-palustris—1
Haematopinus eurysternus—17

ORDER PERISSODACTYLA

Horse—1 examined.

Anocentor nitens—1

Mule—2 examined.

Rhipicephalus sanguineus—2

ORDER XENARTHRA

Armadillo, *Dasypus novemcinctus*—3 examined.
Amblyomma inornatum—2

All ectoparasites were sent to Austin on wet ice for identification and the inoculation of laboratory animals upon which the determination of the presence of *C. burnetii* has been largely based. The guinea pig, hamster and albino mouse have been used as test animals. In many respects the hamster has proven most suitable. A similar conclusion has been reached by Lennette et al. (1951).

A specific complement-fixation test has been done on the blood of both laboratory test animals and many animals examined in the field. The antigen used is prepared principally from the Henzerling strain of *C. burnetii* as produced commercially. Tests are performed qualitatively with a 1 : 10 dilution of the inactivated serum. Except for guinea pig sera which are inactivated at 56° C. for 30 minutes, all sera are inactivated at 60° C. for 20 minutes. Any serum which reacts in the qualitative test is subjected to a quantitative test. A serum control is set up for each test and full sets of controls are included with each run. An overnight procedure similar to that used in the Kolmer modification of the Wasserman Test is utilized.

Occasionally, complement-fixing titers have been noted in pack rat (*Neotoma*) sera taken on the "W.C." ranch, thus focusing attention on the possible importance of this animal in the epizootiology of Q fever. However, *C. burnetii* has not been recovered from the tissues of naturally infected rodents. Several pack rats were trapped alive, bled, marked for identification and released in areas where animals with high complement-fixing titers were found. Some of the released rodents were trapped and bled a second and third time. No instance of a rising titer which would be of possible diagnostic significance has been encountered.

When packrats were inoculated intraperitoneally with small amounts of infective milk, somewhat variable serological responses have been obtained. In one instance there was a surprising delay in the rise in titer. *Ornithodoros talaje,* a common soft-shell tick in Texas pack rat nests, which were allowed to feed on experimentally infected pack rats, acquired the infection between the 6th and 11th day after inoculation of the rodents.

During the portion of the project upon which this study is based, 158 pools of ticks, 79 pools of fleas and 32 pools of lice and mites were inoculated into laboratory animals. All of the ectoparasites were negative* including several pools of *Otobius megnini* which were taken from 2 cows in the "W." dairy herd which were proven shedders of *C. burnetii.* Milk from cows #9 and #25 were repeatedly found to be positive. Field-collected "lone-star" ticks, *Amblyomma americanum,* were allowed to engorge on these cows and their calves on several occasions. Infection was not demonstrable in the ticks when they were subsequently fed on or inoculated into laboratory animals. All 4 quarters of the udder of #25 were shown to be infective. Cow #9 was not so fully investigated. The only negative test on 24 milk samples collected from these cows at various times over the period of a year concerned cow #9 shortly after the birth of a calf. Both cow and calf were found to have high complement-fixation titers at that time. The placental tissue from cow #9 was found not to be infective although Luoto and Huebner (1950) report the isolation of Q fever organisms from placentas of naturally infected dairy cows in California.

* During more recent studies an over-all pool of ectoparasites has been shown to be positive. Individual pools are now being studied to determine the species involved.

SUMMARY

Q fever studies were conducted in Zavala County, Texas, March 1950 to February 1951 in an area in which the disease was shown to be prevalent. A total of 2,261 animals, representing 8 orders, 16 families and 28 species, were examined for ectoparasites and bled for serological studies. Thirty four species of ectoparasites, totaling 9,320 specimens, were taken as listed. The ectoparasites were pooled by species and inoculated into laboratory animals. All of the pools were negative during the study period on which this report is based.

Complement-fixing titers have been shown in pack rat (*Neotoma micropus*) sera.

Ornithodoros talaje ticks allowed to feed on experimentally infected pack rats acquired the infection between the 6th and 11th day after inoculation of the rodents.

Two cows from the "W." dairy were shown to be shedding *Coxiella burnetii* in their milk during the year on which this report is based.

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KOHLSIA WHARTONI AND *STENOPONIA PONERA,* NEW SPECIES OF FLEAS FROM NORTH AMERICA*

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During a survey of trombiculid mites in Mexico, conducted with the support of the Guggenheim Foundation, Dr. George W. Wharton, of the Department of Zoology of Duke University, collected some interesting fleas from the indigenous rodents. Among these was a new species of the genus *Kohlsia* Traub, 1950, which is described at the present time. Also included in this paper is a description of a new species of *Stenoponia* Jordan and Rothschild, 1911, which occurs in Mexico and in the southwestern United States. This is the second species of *Stenoponia* known in the new world.

Kohlsia whartoni n. sp. (figs. 1–11)

Types: Holotype male and allotype female (field number GWW 122) ex *Peromyscus;* Mexico: Vera Cruz, Texolo, coll. G. W. Wharton, 18 October 1950; deposited in Chicago Natural History Museum. A pair of paratypes with same data in United States National Museum, the British Museum and the collection of the senior author. A total of six males and six females with above data (R.T. #8320). One paratype male with same data but collected at Jalapa, 13 October. One paratype male with same data as holotype but with field number GWW 119.

Diagnosis: Distinctive in the relative hyperdevelopment of the male eighth sternum (fig. 6, *8S.* and fig. 11), which is longer and broader than in the known species, and which bears a subapical patch of about ten very long thin bristles. Close to the genotype, *K. osgoodi* Traub, 1950, especially in the structure of the aedeagus. Readily separable as follows: digitoid (fig. 3, *F.*) with but three mesal marginal stout bristles, lacking the ventromarginal one of *K. osgoodi;* female seventh sternum (fig. 8, *7S.*) with sinus broader and deeper, its ventral lobe extending as far caudad as dorsal lobe, instead of dorsal lobe extending more caudad than ventral one.

Description.

Head, Male (fig. 1): Frontoclypeal margin evenly rounded except for distinct, acute tubercle near midpoint; micropunctations scattered above and in front of first row of bristles on both pre- and postantennal regions. Preantennal area with four complete rows of bristles, the first two irregular in number and arrangement; these rows arranged approximately as follows (from front to rear): in male, 6(to 8)–5(to 7)–4–3; in female, 6(7)–4(to 6)–4–3. Uppermost bristle of last row inserted just anterior to eye. Eye distinct, well sclerotized but rather small, about one and one-half times as long as broad; subovate. Genal process broad, somewhat rounded. Maxillary lobe extending slightly beyond apex of third segment of maxillary palpus. Labial palpus five-segmented, extending about five-sixths length of forecoxa. First antennal segment about twice as long as broad; with a marginal row of small bristles and a few proximal and dorsomarginal ones; second antennal segment with a fringe of short bristles which are only about twice as long as this segment. A row of small hairs bordering dorsal margin of antennal fossa. Postantennal region with three rows of bristles, arranged in male approximately 4–4–5(6); occasionally with one or two small extra bristles in first row; in female, these rows arranged approximately 5–6–6(to 8), including 2 at dorsocaudal angle of antennal fossa; in both sexes intercalary hairs between bases of last row usually missing, at times very small; ventralmost bristle of last two rows longer than others in row.

Thorax: Pronotum with one row of bristles; ventralmost bristle longest; with two small intercalary hairs above ventralmost bristle; with one intercalary between others in row; with

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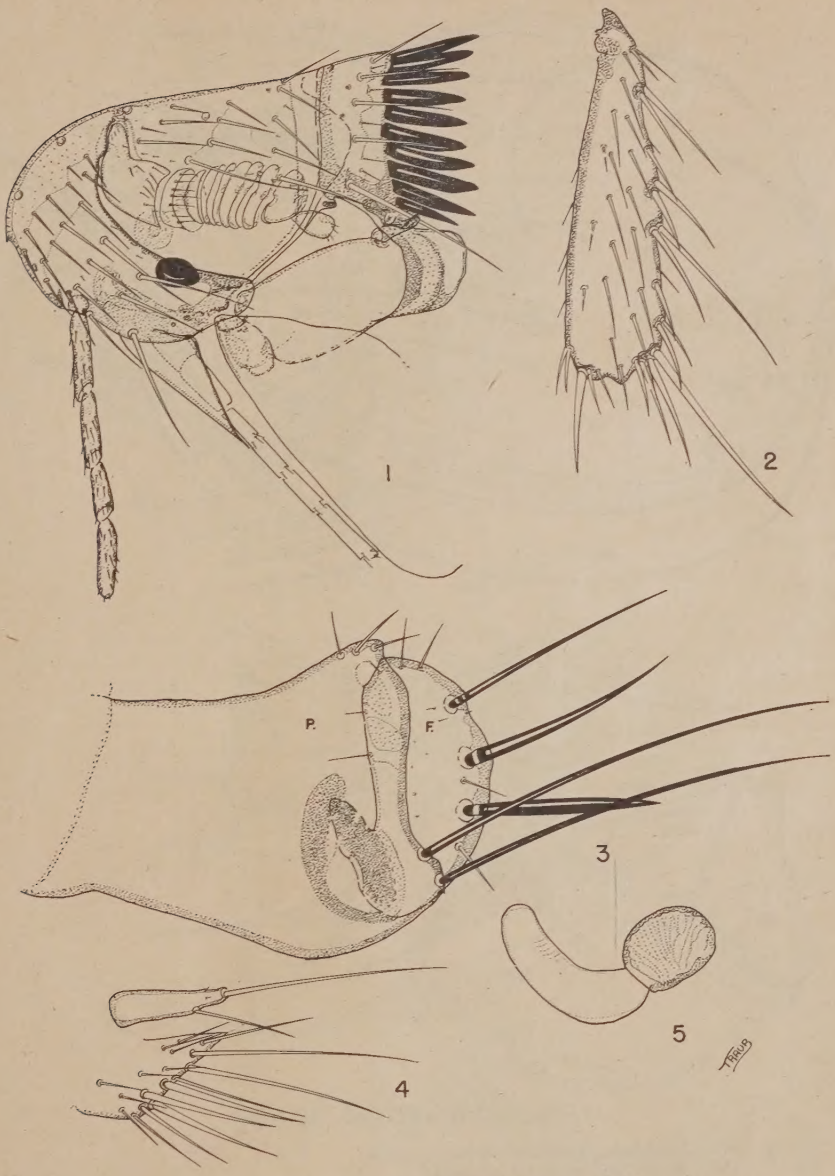
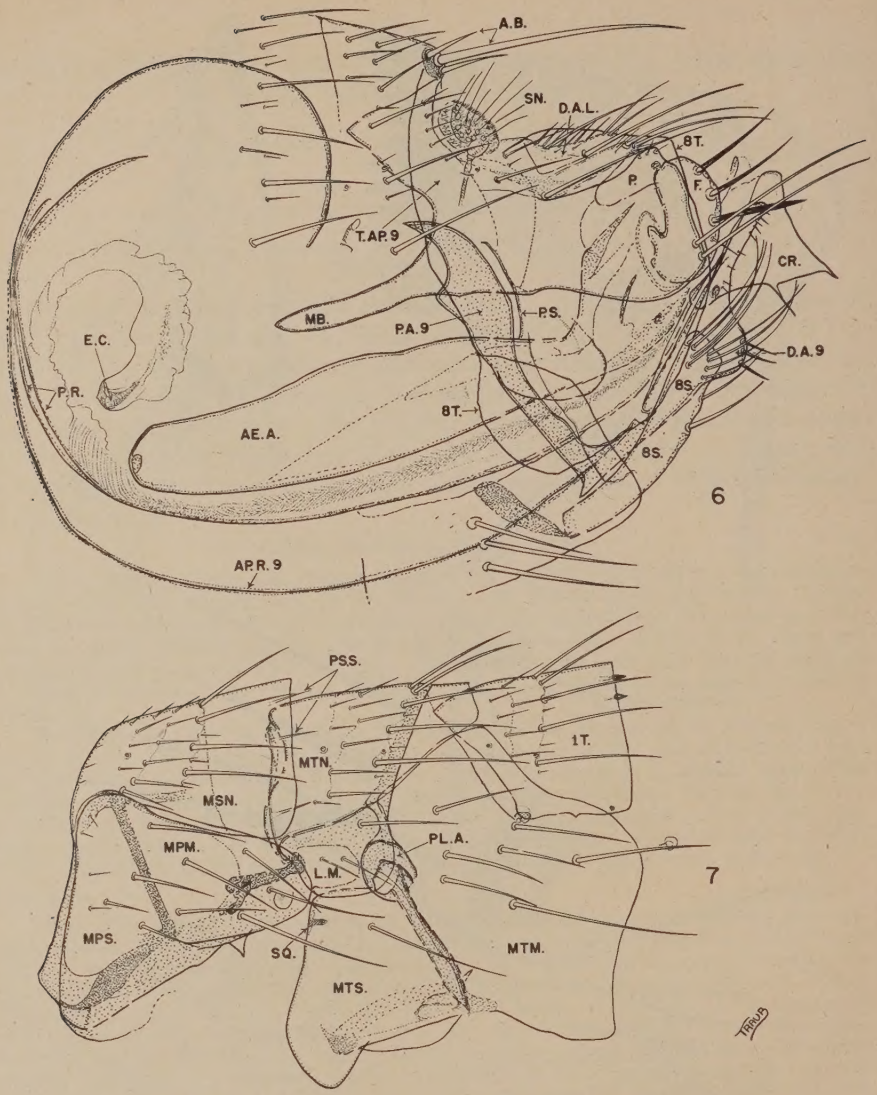


KOHLSIA WHARTONI SP. NOV.

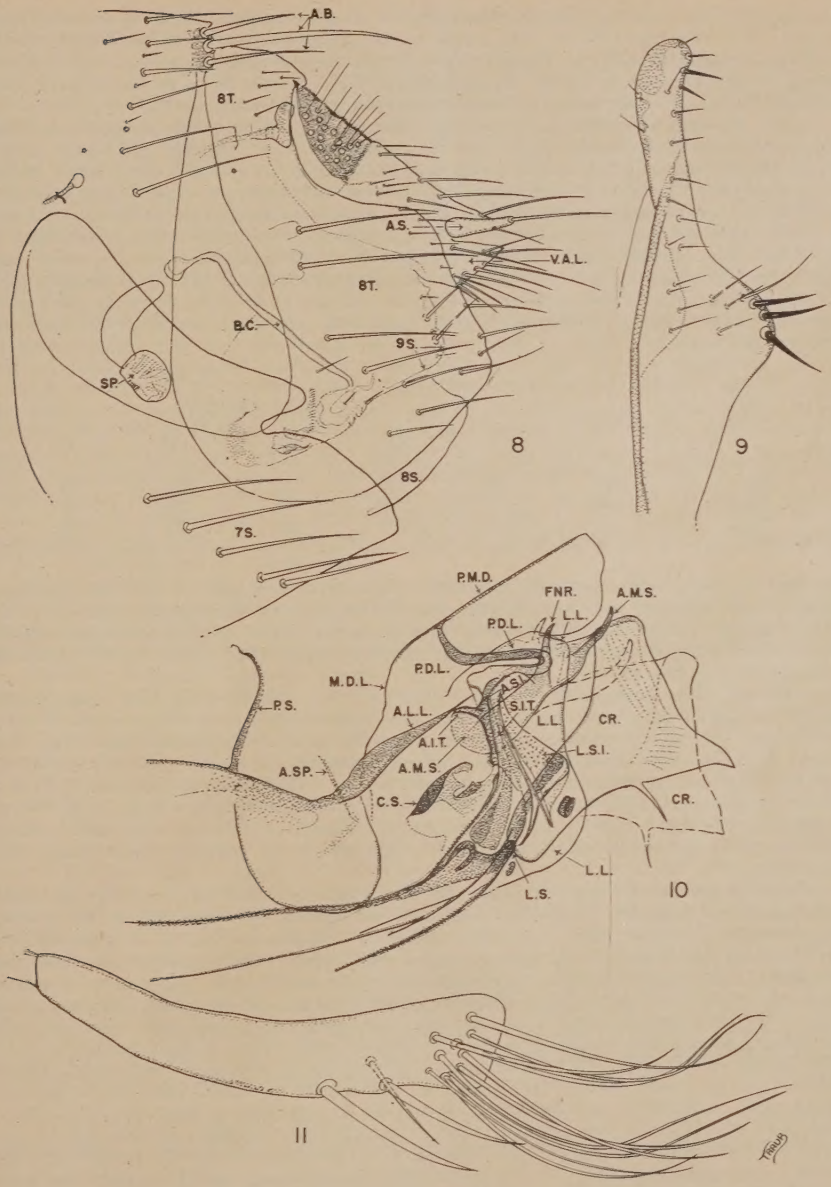
FIG. 1. *Kohlsia whartoni* sp. nov., head and pronotum, male.
FIG. 2. *Ibid.* Metatibia.
FIG. 3. *Ibid.* Process and movable finger (digitoid) of clasper.
FIG. 4. *Ibid.* Anal stylet and ventral anal lobe of female.
FIG. 5. *Ibid.* Spermatheca.



KOHLSIA WHARTONI SP. NOV.

FIG. 6. *Ibid.* Modified abdominal segments, male.
FIG. 7. *Ibid.* Meso- and metathorax, male.

comb of about ten or eleven well-developed spines on a side. Mesonotum (fig. 7, *MSN.*) with three rows of bristles, those of last row longest and with small intercalaries; flange of mesonotum with two or three mesal pseudosetae (*PS.S.*) per side. Mesepisternum (*MPS.*) usually with three medium-sized bristles, two of which are caudomarginal, preceded by two or three smaller lateromedian bristles. Mesepimere (*MPM.*) usually with six or seven bristles arranged 3(4)–3. Metanotum (*MTN.*) with three rows of bristles, the first incomplete or interrupted; those of posterior row longest and with intercalaries. Metanotal flange usually with one subdorsal apical spinelet. Lateral metanotal area (*L.M.*) distinct; with caudal margin lateral to



KOHLSIA WHARTONI SP. NOV.

FIG. 8. *Ibid.* Modified abdominal segments, female.
FIG. 9. *Ibid.* Distal arm of ninth sternum.
FIG. 10. *Ibid.* Endchamber of aedeagus.
FIG. 11. *Ibid.* Eighth sternum.

and extending over much of pleural arch; with two or three bristles, that in dorsocaudal angle longest. Metepisternum (*MTS.*) with a long, submedian bristle inserted at about level of well-developed squamulum (*SQ.*). Pleural ridge of metasternosome fitting into a well-developed socket, the pleural arch (*PL.A.*). Metepimere (*MTM.*) usually with six or seven bristles, arranged 2(3)–3–1; spiracle roughly broadly saggitate, but with apex subrounded.

Legs: Procoxa with many lateral bristles scattered over entire length of segment. Meso- and metacoxae with few such bristles and those submarginal or subapical. Profemur with a dorsomarginal row of small bristles; with a long bristle at dorsocaudal angle; with two or three subapical and two or three submarginal bristles; with about six small lateral submedian bristles; with one subapical ventromarginal bristle. Mesofemur essentially similar, but with pair of bristles at dorsocaudal angle; frequently with one small apical mesal bristle. Metafemur as mesofemur but with about five to seven submarginal, dorsal small bristles; with one or two small bristles near the large one at anteroventral angle. Metatibia (fig. 2) with large dorsomarginal notches bearing large bristles as follows (from base to apex): 2–1–2(1)–2–1–2 (excluding the apical 3). Measurements of tibiae and segments of tarsi (petiolate base deleted) of holotype shown in microns:

Leg	Tibia	Tarsal segments				
		I	II	III	IV	V
Pro-	160	75	60	55	45	90
Meso-	280	135	110	75	50	95
Meta-	375	290	200	125	75	115

Pro- and mesotibiae with one bristle which extends to or beyond apex of first tarsal segment. None of tarsal segments with apical bristle extending beyond apex of succeeding segment. First tarsal segment in each case with proximal pair of plantar bristles slightly displaced medially, and with four pairs of lateral plantar bristles. Blade of unguis about twice length of thickened recurved basal portion.

Abdomen: First tergum (fig. 7, 1T.) with three rows of bristles, the first represented by but two bristles; those of last row longest and with intercalaries; flange with two small apical spinelets per side. Terga two through five usually with one or two apical teeth per side. With second row of bristles extending ventrad to subovate spiracle. Basal sternum with one ventromarginal bristle. Unmodified sterna usually with row of three bristles in males, with three or four in females; male with three antepygidial bristles (*A.B.*); median one long; other two reduced but ventralmost slightly longer than dorsal. Female with three antepygidial bristles (*A.B.*); middle one nearly twice length of others and with lower bristle somewhat longer than uppermost.

Modified Abdominal Segments, Male (fig. 6): Eighth tergum (*8T.*) very well developed, covering most of genitalia. With about four subdorsal bristles, the posteriormost two of which are longest, and at times with a more median, lateral long bristle. Eighth sternum (*8S.* and fig. 11) about five times as long as broad; apex subtruncate; distally somewhat broader than proximally; with two fairly stout ventromarginal bristles and a patch of about ten very long subapical bristles; at times with a submedian mesal or lateral bristle above ventromarginals. Base of developed portion of eighth sternum associated with a fairly well sclerotized and semimembranous section which extends dorsad to level of penis rods. Clasper essentially as in other species of the genus. Immovable process (*P.* and fig. 3) with three small apical bristles and two long acetabular bristles; latter marginal and inserted on ventral convexity of posterior margin of *P.* Digitoid or movable finger (*F.* and fig. 3) extending to near apex of *P.*; somewhat more than twice as long as broad; anterior margin fairly straight, posterior margin convex; apex broadly rounded; with three submarginal very stout mesal bristles or subspiniforms; lowest of these inserted at level of dorsal margin of acetabulum; with two small thin apical mesal bristles, a similar one above and below ventral subspiniform and two along anterior margin; with a submedian row of very small bristles. Tergal apodeme of ninth segment (*T.AP.9*) about equal in length to that of narrow manubrium (*MB.*). Ninth sternum with proximal arm (*P.A.9*) about as long as distal arm (*D.A.9*). Proximal arm broadened apically and here arched like a crooked finger. Distal arm of ninth sternum (*D.A.9* and fig. 9) with a prominent convexity on caudal margin, near middle; this lobe bearing three short marginal subspiniforms, three submarginal mesal thin bristles and one thin lateral bristle. Distal arm long and narrow apicad of lobe, with three short fairly stout apical or subapical bristles and two anteromarginal bristles; with a patch of scattered thin lateromedian bristles extending down to level of lobe.

Distal arm with a dorsal sclerotized portion which extends distad as a mesal elongate, acuminate projection reaching to near apex of arm.

Aedeagus: Aedeagal apodeme (*AE.A.*) about twice length of aedeagus proper; more than three times as long as broad; dorsal margin slightly sinuate. Proximal spur (*P.S.* and fig. 10) well developed. A somewhat similar accessory spur (*A.SP.*) at base of aedeagus arising from near base of tongue-like, proximally broad, apically acuminate accessory lateral lobe (*A.L.L.*). Median dorsal lobe (*M.D.L.*) greatly expanded; flared apically, convoluted, forming a primary median dorsal lobe (*P.M.D.*) and a secondary paradorsal lobe (*P.D.L.*). Lateral lobes (*L.L.*) well developed, sinuate; extending dorsad to paradorsal lobe. Associated with dorso-apical corner of lateral lobes, and with paradorsal lobe, a prominent acuminate spur extending on each side distad of apex of inner tube; this spur (*FNR.*) mesad to lateral lobes but laterad to inner tube; probably analogous if not homologous with the structure referred to as the fender (1). Crochet very well developed, its well-sclerotized portion almost as broad as long; with a fang-like projection of ventral margin, distad of midpoint; sclerotized portion of crochet proximad of fang about twice as long as broad. The apparent dorsal margin of crochet strongly curved dorsad distad of midpoint; dorsal margin subtruncate; anterior margin slightly concave; ventrocaudal angle somewhat acuminate. Sclerotized inner tube (*S.I.T.*) subvertical; heavily armored, its apex (*A.S.I.*) curved dorsocaudad, sclerotized portion extending apicad of the aperture as a narrow band. Armature of inner tube (*A.I.T.*) apparent as an acuminate anterior-directed projection at apex of sclerotized inner tube; another portion of this armature represented as a lateral sclerotization (*L.S.I.*) flanking the caudal portion of the vertical sclerotized tube; apical portion of inner tube on each side flanked by a long sclerite (*A.M.S.*) which terminates in an elongate acuminate projection, extending to dorso-apical margin of crochet. Apodemal strut well developed, as is its lateral ventral lobe (*L.S.*); crescent sclerite (*C.S.*) conspicuous; penis rods (*P.R.*) not coiled. With a characteristic cap-shaped sclerotization (*E.C.*) at proximal end of endophallus.

Tenth abdominal segment conspicuous; sensilium fairly flat; dorsal lobe of proctiger (*D.A.L.*) with a dorsomarginal row of bristles and a few lateromedian bristles; longest bristles apical; ventral lobe of proctiger (*V.A.L.*) about three times as long as broad; with a few subapical and long apical bristles; proximal ventral sclerite represented by a subtriangular dark area.

Modified Abdominal Segments, Female (fig. 8): Seventh sternum (*7S.*) with a narrow sinus so that resulting dorsal lobe is narrow and subacute; ventral lobe broadly rounded; with a row of about five bristles. Eighth tergum with about seven short bristles dorsad to spiracle; two long bristles ventral to sensilium; five long or fairly long lateromedian bristles; about three subventrals and three to five caudomarginals; with two mesal short bristles at level of ventral anal lobe. Eighth sternum (*8S.*) somewhat longer than broad, lightly sclerotized. Ninth sternum (*9S.*) weakly sclerotized, with outlines still partially apparent; with a few small slender bristles along caudal margin, apparently lateral to proximal portion of ninth sternum, with a spiculose area on each side. Dorsal anal lobe of proctiger with marginal bristles and about eleven scattered lateral or lateromedian bristles; in addition with four or five marginal bristles below anal stylet. Anal stylet (*A.S.* and fig. 4) about two and one-half times as long as broad at base; with a long apical bristle and a shorter ventromarginal bristle; with one or two vestigial subapical bristles. Ventral anal lobe (*V.A.L.* and fig. 4) angulate, short; caudal margin with three stout bristles above angle; with subapical long thin bristles; with two or three smaller ones at angle; with about seven small submarginal bristles. Spermatheca (*SP.* and fig. 5) with head about four-fifths as broad as long; dorsal margin fairly convex, ventral margin almost straight; with tail upturned and much longer than head; relatively dilated. Bursa copulatrix (*B.C.*) with dorsal end dilated; fairly well sclerotized; the duct slightly arched proximad of head and then fairly straight and somewhat sclerotized.

Comments: This species is named for the collector, Dr. George W. Wharton, who is one of the foremost students of trombiculid mites and other ectoparasites.

The aedeagus of *K. whartoni* sp. nov. (fig. 10) is essentially like that of the genotype *K. osgoodi* Traub 1950, not only in the flaring convoluted median dorsal lobe and in the general shape and fang-like extension of the crochet, but also as follows: (a) the presence of an acute spur-like fender (*FNR.*) associated with the lateral lobe (*L.L.*) and the primary fold of the median dorsal lobe (*P.M.D.*), on each side of the apex of the inner tube (*A.S.I.*); (b) the conspicuous acuminate rod-like extension of the apicomedian sclerite (*A.M.S.*) flanking the inner tube,

forming what was originally referred to as the "pseudolobe;" (c) a sclerotized cap (fig. 6, *E.C.*) at the proximal end of the endophallus.

Heretofore six species of the genus *Kohlsia* were known (Traub, 1950). Five of these species were described from specimens collected in Guatemala, Nicaragua or El Salvador. The sixth, from Ecuador, is known only from the female, and may really belong to another genus. The description of *K. whartoni* bears out the original prediction of the occurrence of this genus in Mexico, while an undescribed species from Panama suggests that the genus indeed may be found throughout northern South America. These fleas all seem to parasitize small rodents such as mice (especially *Peromyscus*) and tree squirrels, as do the related *Pleochactis* Jordan, 1933 and *Jellisonia* Traub, 1944. Relatively little is known about these fleas, as evidenced by the fact that six of the eleven species of *Pleochaetis* have been described since 1949, while all of the seven in *Jellisonia* have been described since 1943. Further, an undescribed species of each of these genera is in the collection of the senior author. These fleas include a relatively large number of species and are quite common, and, because of their host preferences, it is quite likely that the genera *Kohlsia*, *Pleochactis* and *Jellisonia* may prove to be of medical significance.

Stenoponia ponera n. sp. (figs. 12–21)

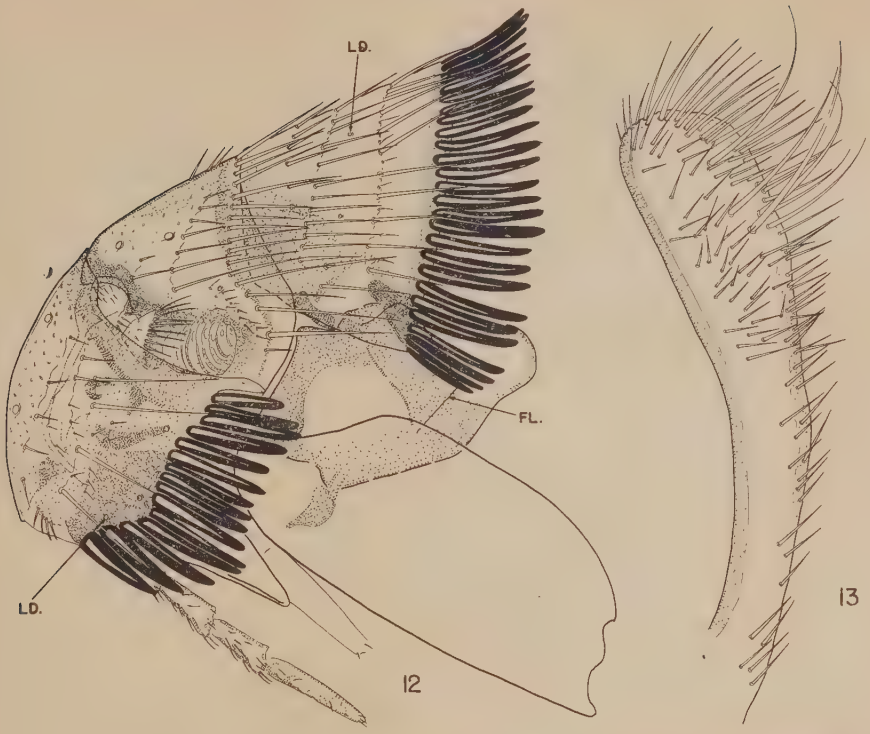
Types: Holotype male, allotype female, four male and one female paratypes ex *Peromyscus boylii;* New Mexico: Grant County, 7 miles north of Pinos Altos, elev. 6900 ft., 12 October 1950, N.M. 276, coll. Public Health Service plague survey team. Two paratype males with same data but ex *Eutamias dorsalis;* two paratype males and one paratype female ex *Peromyscus boylii*, with same data except N. M. 277. One paratype male ex *Peromyscus*, Mexico: Durango, Laguna del Progresso, 27 June 1950, coll. Paul S. Martin. One paratype female found in collection of spiders received by American Museum of Natural History from Mexico: Mexico, D. F., December 1944, coll. H. Wagner. Holotype and allotype deposited in collections of United States National Museum (No. 61,294). Paratypes deposited in collections of Chicago Natural History Museum, the Western Communicable Disease Center Laboratories, Public Health Service, San Francisco, California, the American Museum of Natural History, and in the collection of the senior author.

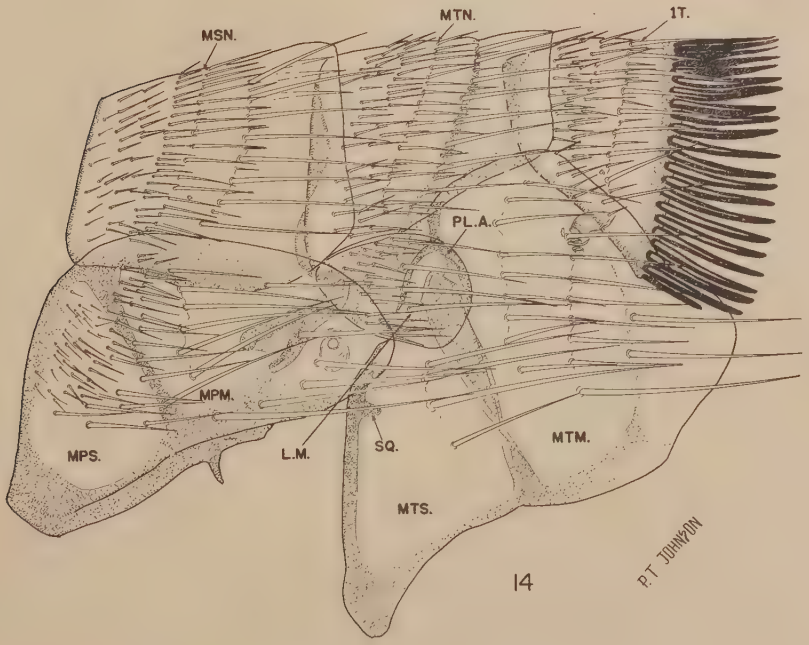
Diagnosis: May be separated from *Stenoponia americana* (Baker, 1899) by the following characters: Preantennal region with two distinct rows of bristles (fig. 12), not one. Postantennal region with marginal row complete, extending from apex of antennal groove to top of head, not with marginal row extending merely to level of base of first antennal segment; first row consisting of five bristles, not one or two. Pronotum with two and one-half rows of bristles, not with four complete rows. Crochet (*CR.*, fig. 15 and fig. 17) hook-shaped, narrow, not broad and talon-shaped. Hood of aedeagus (*H.*, fig. 15 and fig. 17) with definite dorso-apical hump, not smoothly convex. In female horizontal margin of seventh tergum (fig. 20, *7T.*) arising immediately below base of antepygidial bristles, not produced into an acuminate lobe. Spermatheca (*SP.*, fig. 19 and fig. 20) body with flange surrounding base of tail, not lacking flange. Eighth tergum with row of bristles extending dorsally almost to level of spiracle, not ending mediolaterally, far below spiracle. Female with six antepygidial bristles, not five.

Description.

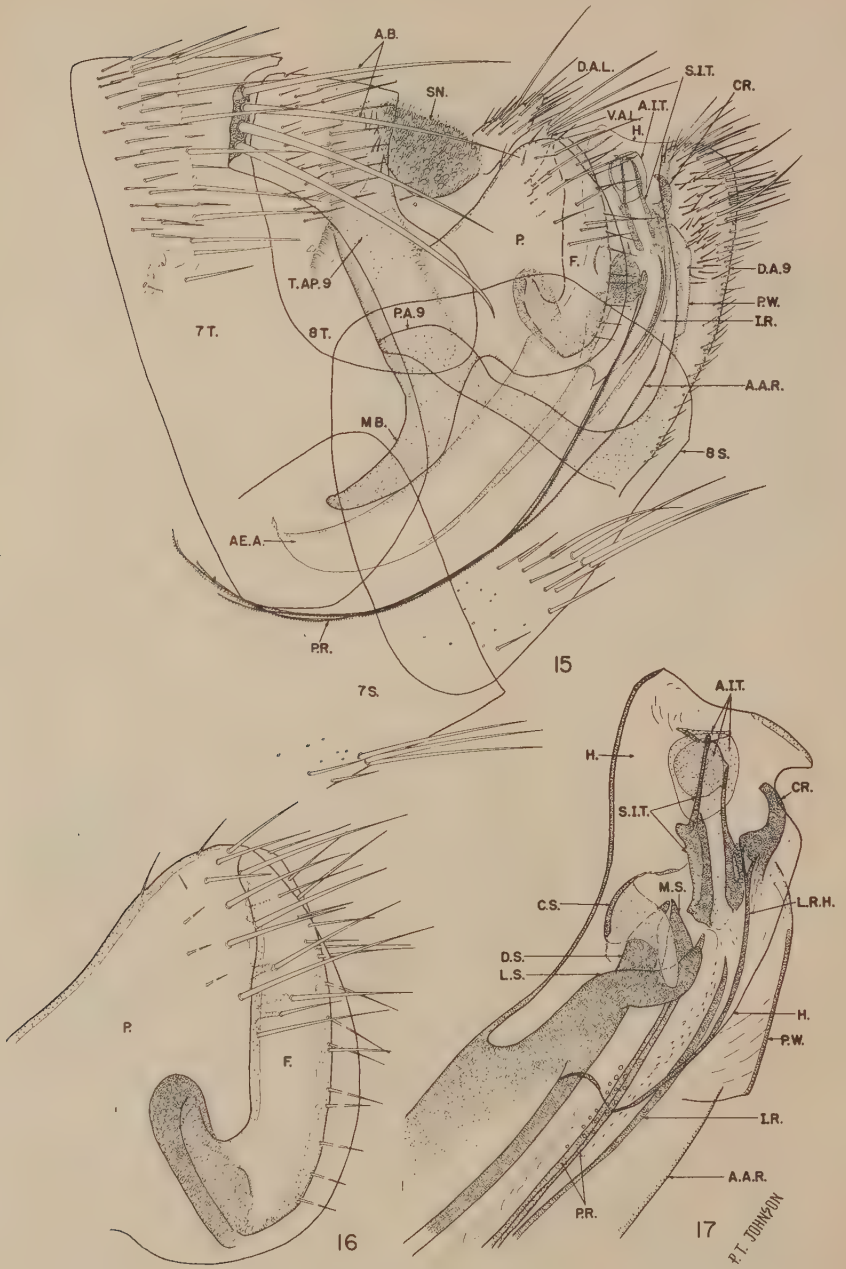
Head, Male (fig. 12): Preantennal area with two rows of bristles, upper of six small bristles, lower of five or six larger ones, one or two nearest antennal groove relatively shorter; several small hairs scattered over area between rows of bristles and also ventral to lower row. Numerous micropunctations above bristles on pre- and postantennal areas. Tentorial arm evident. Genal comb of fourteen or fifteen teeth on a side, slightly sinuate and subhorizontal in position; head margin in this region with three clear, raised subspherical bodies, perhaps sensory in function and here referred to as lucodiscs (*LD.*). Labial palpus three-segmented, ex-

FIG. 12. *Stenoponia ponera* sp. nov., head and prothorax, male.
FIG. 13. *Ibid.* Distal arm of ninth sternum.
FIG. 14. *Ibid.* Meso- and metathorax, male.



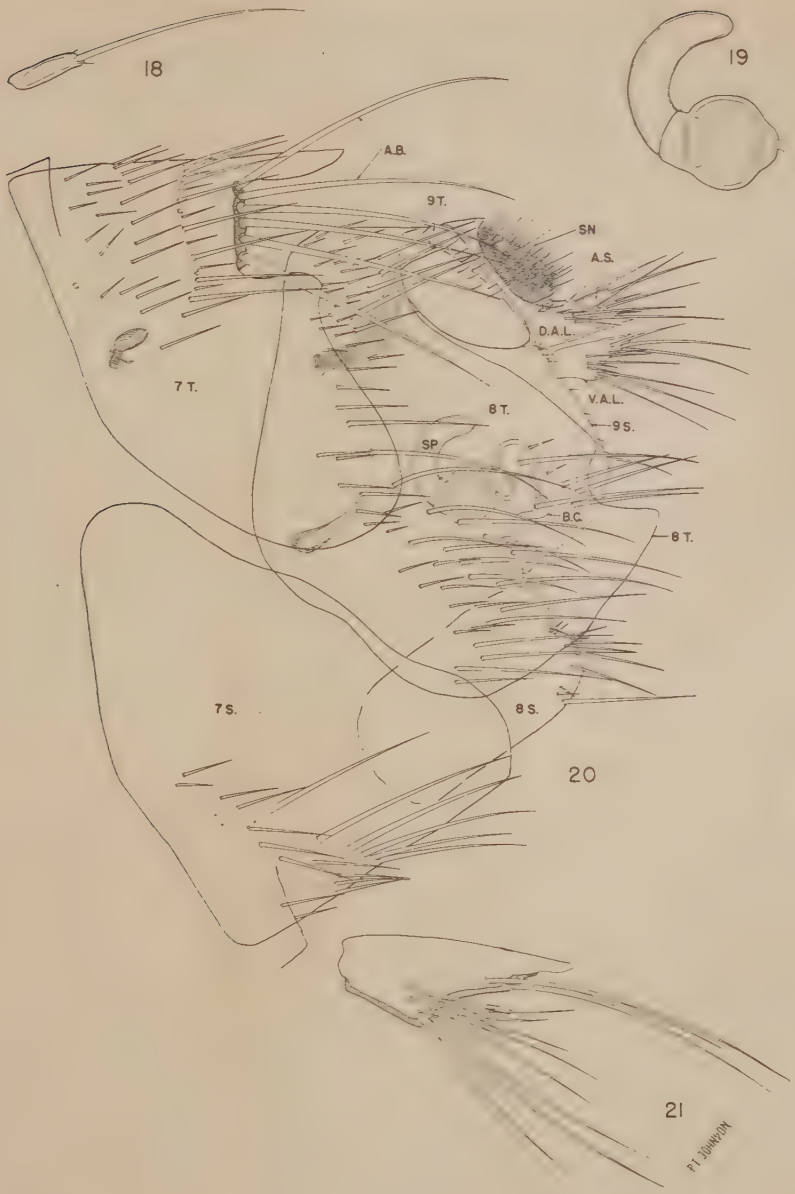


STENOPONIA PONERA SP. NOV.



STENOPONIA PONERA SP. NOV.

FIG. 15. *Ibid.* Modified abdominal segments, male.
FIG. 16. *Ibid.* Immovable process and digitoid of clasper.
FIG. 17. *Ibid.* Apical portion of aedeagus.



STENOPONIA PONERA SP. NOV.

Fig. 18. *Ibid.* Anal stylet, female.
Fig. 19. *Ibid.* Spermatheca.
Fig. 20. *Ibid.* Modified abdominal segments, female.
Fig. 21. *Ibid.* Ventral anal lobe.

tending about one-half length of forecoxa. First antennal segment with several small bristles lateromedially, and a row of short bristles apically; second antennal segment with two rows, bristles of apical row not reaching apex of club in either sex. Postantennal area with an anterior oblique row of five or six bristles; above row, one or two small bristles near antennal groove; a sinuate row of seventeen or eighteen long bristles along entire posterior margin of head; most of dorsal bristles displaced to near margin of flange; several short bristles along posteroventral margin of antennal groove.

Thorax: Pronotum with a comb of twenty-six to twenty-eight spines on a side and with two complete rows of bristles plus three or four bristles in an irregular anterior row; small intercalaries in most posterior row, three lucodiscs scattered below first and between second and third rows; prosternum with a lightly sclerotized lateral flange (fig. 12, *FL.*) extending over base of most ventral spines of pronotal comb, margin of flange rectangular. Mesonotum (fig. 14, *MSN.*) densely clothed with about six rows of bristles, those of last two rows longest, last row with intercalaries; with six subdorsal pseudosetae on mesal surface of flange. Mesepisternum (*MPS.*) clothed with short bristles; anteriorally, about six longer bristles at posteroventral angle. Mesepimere (*MPM.*) with one vertical row of six long bristles, a semicircular row of six to eight long bristles following contour of posterior and ventral margins, and with patch of about fifteen short bristles near upper anterior margin. Lateral metanotal area (*L.M.*) present, covering pleural arch; with scattered small bristles anteriorally; two rows posteriorally, first of about eight, second of four or five; bristles of posterior row long. Pleural arch (*PL.A.*) short but distinct. Metanotum (*MTN.*) with four rows of bristles, bristles of most posterior row longest and with intercalaries. Metepisternum (*MTS.*) with four long bristles and well-developed squamulum (*SQ.*); metepimere (*MTM.*) with three rows of long bristles arranged 8(9)–8–2.

Legs: Procoxa with many lateral bristles; meso- and metacoxae with bristles confined to anterior margin. Profemur with about nine dorsomarginal bristles, twenty-three lateral bristles, one ventrobasal bristle, two mesal bristles, two ventral subapical bristles and two long apical bristles. Mesofemur similar but with fourteen dorsomarginal bristles; four or five mesal bristles; two ventrobasal bristles; lateral bristles forming row from base; with most of upper anterior region bare; with three ventral subapical bristles. Metafemur as mesofemur but with eleven dorsomarginal bristles and four ventral subapical bristles. Protibia with longest bristle reaching apex of second tarsal segment; meso- and metatibia with longest bristle reaching apex of first tarsal segment. Measurements of tibiae and tarsal segments (excluding petiolate base) of holotype shown in microns:

Leg	Tibia	Tarsal segments				
		I	II	III	IV	V
Pro-	391	165	113	82	62	165
Meso-	484	391	185	103	82	155
Meta-	773	649	381	227	113	175

Four pairs of lateral plantar bristles on fifth segment of all tarsi, one median pair between basal lateral pair.

Abdomen: First tergum (fig. 14, *1T.*) with four rows of bristles, those of most posterior row longest and with intercalaries; posterior margin with comb of twenty to twenty-two spines on a side (twenty-five in female). Terga two to six with apical spinelets as follows: (male) second tergum 20–29, third tergum 16–25, fourth tergum 15–23, fifth tergum 6–16, sixth tergum 0–5; (female) second tergum 20–26, third tergum 19–22, fourth tergum 15–22, fifth tergum 6–15, sixth tergum 0; spinelets extending about to level of spiracle. Typical abdominal terga with four rows of bristles, posterior row with intercalaries and extending ventrad to spiracle. Basal abdominal sternum with three or four ventromarginal bristles (one in female); unmodified sterna usually with three short rows of ventromarginal bristles arranged 1(2)–3–3(4). Male with four long subequal antepygidial bristles, female with six.

Modified Abdominal Segments, Male (fig. 15): Eighth tergum (*8T.*) small, extending ventrad to level of base of manubrium of clasper; with three dorsolateral rows of bristles ending at base of spiracle, those of most posterior row longest. Dorsoposterior margin of eighth sternum (*8S.*) with a posterior row of three or four long bristles preceded by several small scattered bristles and three or four ventromarginal bristles. Process of clasper (*P.* and fig. 16) with seven or eight long bristles lateromedially, forming double row from apex to level of dorsal margin of acetabulum; a few short apical and subapical bristles, several short mesal bristles; apex rounded, posterior margin delicate, evenly convex. Manubrium (*MB.*) less than length

of *P.*, fairly broad, becoming acuminate. Tergal apodeme of segment nine (*T.AP.9*) with straight anterior margin, about as long as manubrium.

Digitoid or movable finger (*F.* and fig. 16) almost reaching apex of process *P.* of clasper; more than four times as long as broad, with fourteen or fifteen bristles on posterior margin, some of these longer than width of digitoid; acetabulum set medially in process, so that most of *F.* is overlapped by *P.*

Proximal arm of ninth sternum (*P.A.9*) narrow, slightly expanded dorsoapically, with subacuminate tip; almost as long as distal arm. Distal arm (*D.A.9* and fig. 13), club-shaped, with narrow basal portion rapidly expanding into rounded truncate apex; apical half heavily covered with bristles; with row of marginal and submarginal bristles from base to apex; five or six long stout bristles in apical clump.

Aedeagus (figs. 15 and 17): Aedeagal apodeme (*AE.A.*) about one and one-half times length of aedeagus proper; six times as long as broad. Hood (*H.*) with rather narrowly rounded apex, posterior margin concave directly below apex, posteroventral angle acute, reaching ventrally to below level of crochet and running cephalad beyond end of pouch wall (*P.W.*), where it turns abruptly dorsad. Pouch wall sclerotized on ventral margin, beginning just cephalad of crochets. Crochets (*CR.*) narrow, strongly hooked, with subtruncate apex, base much broader than apex. Sclerotized inner tube (*S.I.T.*) heavy, six times as long as broad, subparallel to dorsal margin of hood. Armature of inner tube (*A.I.T.*) represented by three lightly sclerotized plates, two of which overlie each other, with the third more or less vertical to these at apex of inner tube. Crescent sclerite (*C.S.*) long and narrow, with ventral arms lightly sclerotized and extending over lateral (*L.S.*), median (*M.S.*) and dorsal (*D.S.*) sclerites of apodemal strut. A long narrow lateral thickening of ventral portion of hood, here referred to as lateral rib of hood (*L.R.H.*), extending from, and perhaps connected with, base of crochets to anterior margin of hood in vicinity of apodemal strut, where it becomes a sclerotization of hood margin. Penis rods (*P.R.*) extending only slightly beyond apex of aedeagal apodeme. Aedeagal apodemal rod (*A.A.R.*) with apex lying free just within pouch. Intramural rod (*I.R.*) extending caudad to level of apodemal strut, and cephalad about one-half length of aedeagal apodeme.

Sensilium (fig. 15, *SN.*) flat, with several very small bristles along anterior border. Dorsal anal lobe of proctiger (fig. 15, *D.A.L.*) with dorsomarginal bristles; one long subapical bristle; several lateral bristles on apical half. Ventral anal lobe (fig. 15, *V.A.L.*) with three long apical bristles and two or three small slender bristles at posteroventral angle.

Modified Abdominal Segments, Female (fig. 20): Seventh sternum (*7S.*) with slightly sinuate margin; with a row of six or seven long bristles and a row of about eleven shorter bristles preceding this; one or two small bristles anterior to both rows. Seventh tergum (*7T.*) with caudal extension dorsally between antepygidial bristles; posterior margin running caudad from base of antepygidial bristles, then turning vertically, forming a right or slightly acute angle. Eighth tergum (*8T.*) with row of about six rather stout bristles anterior to spiracle, a row of shorter bristles anterior to this, and about ten small scattered bristles preceding first row. Below spiracle eighth tergum with a row of about nine rather long bristles; fifteen or more mediolateral bristles anterior to row of long bristles; two or three long bristles near caudal margin; mesally in this area four or five shorter bristles; about five long sub-basal ventral bristles; posterior margin sinuate to middle, excised deeply at this point, sharply angled posteroventrally. Eighth sternum (*8S.*) about twice as long as broad; with rounded apex; with about five slender subapical bristles and two or three long ventral bristles. Ninth tergum (*9T.*) visible as short sclerite anterior to sensilium and extending ventrad somewhat beyond base of sensilium; with fifteen small slender lateral bristles. Ninth sternum (*9S.*) with several very small posteromarginal bristles. Dorsal anal lobe of proctiger (*D.A.L.*) with several short and long bristles. Anal stylet (*A.S.* and fig. 18) set close to dorsal margin of *D.A.L.*; with one long apical bristle and two very small dorsal and ventral subapical bristles. Ventral anal lobe (*V.A.L.* and fig. 20) with definite hump posteroventrally; marginal bristles usually arranged in two groups: five or six closely set long bristles on hump, this usually associated with six or more submarginal bristles; the second group of about four long subapical bristles; at times with one or two marginal bristles between these groups. Spermatheca (*SP.* and fig. 19) with head subovate or subglobular; flange surrounding base of tail, tail upcurved, twice as long as head. Bursa copulatrix (*B.C.*) with globular portion adjoining spermethecal duct; narrowing from this point; lightly sclerotized.

Comments: It is interesting to note that the genus *Stenoponia* is characterized by a hyperdevelopment of the labral region, which is elongate and bears very fine hairs, and which frequently overlaps part of the mouthparts (cf. fig. 1 and fig. 12).

This unusual condition is even more pronounced in *Stenoponia tripectinata* (Tiraboschi, 1902) and *S. singularis* Ioff and Tiflov, 1933, the only Old World forms available for study.

ACKNOWLEDGMENTS

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AN EFFICIENT METHOD FOR EXPOSURE OF MICE TO CERCARIAE OF *SCHISTOSOMA MANSONI*

LOUIS OLIVIER[1] AND M. A. STIREWALT[2]

In the course of extended studies with infections of *Schistosoma mansoni* in mice we have evolved a simple, safe, and efficient method of exposure which has certain advantages over previously described procedures. It is felt the method may prove useful to workers now studying the schistosomes in other laboratories.

The technique incorporates features of previously described methods of exposure and consists essentially of restraining the mice in the supine position on boards and exposing their tails to a cercarial suspension in small tubes.

Preparation of the cercarial suspension.—The cercariae for the exposure are delivered into glass tubes of the desired size (we have found Kahn tubes 75 × 12 mm. to be most suitable). Known numbers of cercariae may be delivered by two general methods depending on the conditions of the experiment. In the first method cercariae are transferred drop by drop from a capillary pipette to the inner surface of the tube. The number of cercariae in each drop is counted at the time of transfer with the aid of a dissecting microscope. Because it is somewhat tedious and time-consuming, this method is employed only when great accuracy in the counts is necessary and when the number of cercariae used is small.

When large numbers of cercariae per mouse are required they may be delivered into the tubes by aliquot from a stirred suspension of known concentration. This concentration can be determined by delivering a series of aliquots into ruled Syracuse watch dishes and counting the cercariae after they have been killed with formalin. Various types of pipettes are available for transferring the cercariae; we have found the small Alfred Bicknell[3] pipette fitted with a delivery tube having an aperture of about 3 millimeters in diameter to be both useful and accurate. It may be set to deliver from one-fourth to 3 ml. of fluid. This pipette is especially well adapted for delivering suspensions of living cercariae because the large aperture reduces turbulence and associated injury to the cercariae, the cercarial suspension wets only the glass tube and does not enter the bulb, and the glass portions of the pipette can be checked for cercariae that may adhere to its sides.

When the tubes are filled they are arranged in a rack in such a way that mice fixed to small boards may be placed directly above the openings of the tubes with their tails hanging into the cercarial suspension. (Fig. 1.)

Preparation of the mice for infection.—Two persons work together in immobilizing the mice. Each mouse is fastened in the supine position to a thin board (5 by 16 by 0.6 cm.) which has a small hole equidistant from the two long edges and 5 cm. from the end, and a small nail in each corner at the same end. (Fig. 1)

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[3] An automatic pipette with a rubber bulb and long glass delivery tube. Alfred Bicknell Associates, Cambridge, Mass.

(Nolan, Mann, and Churchill, 1947.) A rubber band is fastened to each hind leg of the mouse, and the tail is threaded through the hole in the board. The rubber bands are then fastened to the nails, putting the hind legs under slight tension. A band of adhesive tape one inch wide is then placed over the thorax and fore legs of the mouse, fastening the trunk of the mouse to the board and pinioning the fore legs. Precaution must be taken at this point to avoid interference with breathing. A narrow band of tape is added across the hind legs to reduce their activity and to keep the tail in the hole. After some practice a mouse may be fastened to the board

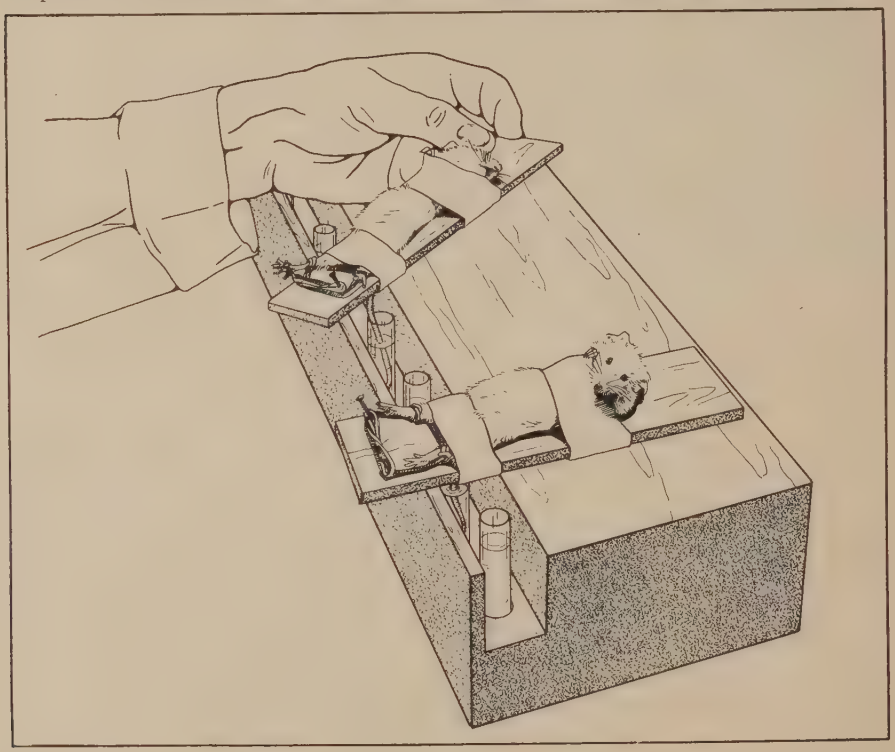

FIG. 1.

in less than 45 seconds. Mice restrained in this way remain unharmed for several hours.

Method of exposure to the cercarial suspension.—After the mice have been fixed to the boards their tails are rubbed with moistened fingers to wet the skin and so increase the chances of effective cercarial contact. The boards are then placed over the tubes in the rack (Fig. 1) in such a way that the tails hang well into the tubes. The exposure period is usually from 30 to 60 minutes. The boards are then removed from the racks and the tails blotted and allowed to dry after which the mice may be freed from the boards and returned to the cages.

DISCUSSION

Various techniques for exposure of mice to *Schistosoma mansoni* have been devised and some have been described (Cram and Files, 1947; Kuntz et al, 1947;

Yolles et al, 1949; Standen, 1949; Watson and Azim, 1949; Hitchcock, 1949; and others). Each technique has certain advantages and disadvantages depending on the experimental design.

Safety and speed are always desirable in any method of exposing mice to cercariae. Often it is necessary to duplicate the natural mode of infection, produce relatively uniform infections, provide a maximal opportunity for cercariae to penetrate, and permit accurate control of the size of the infective dose. Sometimes special considerations demand further refinements. For example, it may be essential to limit the area of host skin exposed or to count the number of cercariae which do not penetrate. The method described here meets these needs.

The method is relatively safe. Cercarial suspensions can be delivered into the tubes with safety, and there is comparatively little subsequent risk to the operator since the mice cannot splash the cercariae out of the container. Moreover, the freshly exposed animals can be handled more freely, since only a small area of the skin has been exposed.

The method is relatively fast. Two persons can expose 100 mice to known numbers of cercariae in 4 hours if the cercariae are measured into the tubes by the aliquot method. Greater precision in the cercarial counts or added detail require

TABLE 1.—*Penetration of cercariae of* S. mansoni *into the tails of mice exposed for varying lengths of time. Exposure of one-fourth of each group of 20 mice was terminated at the end of each 15-minute period and the cercariae still free in the tubes were counted*

	Number of cercariae per mouse (per tube)	Number of mice	Percentage of cercariae apparently penetrating within the given exposure periods. (Cercariae introduced into the tube minus the cercariae found in the tube after exposure period.)			
			15 min.	30 min.	45 min.	60 min.
Group I	200	20	77.7	68.8	90.4	90.0
Group II	100	20	78.6	85.2	89.0	89.6

more time. For example, if it is necessary to count the cercariae into the tubes by the drop method and to check the tubes for cercariae after the exposure period only about 50 mice can be exposed in 4 hours.

The method makes use of the normal route of entry into the host, through the skin. Moreover, the exposed area lacks the barrier of thick hair found elsewhere on the mouse and is a limited area comparable for different exposures.

The method produced infection in every mouse, and the uniformity of infection compared favorably with that obtained by other methods. Moreover, larger numbers of worms came to maturity in the mice when compared with the returns following other described methods of exposure. This is demonstrated in Fig. 2 which compares worm yields from two series of infected mice which differed only in the method by which they were exposed.

Control of the infective dose may be as accurate as the individual investigator desires. Widely different concentrations of cercariae may be used under identical conditions and the number of cercariae remaining after exposure may be determined. An example of the sort of information obtainable by this method is presented in Table 1.

This method, therefore, satisfies the requirements routinely demanded. In addition, it has special advantages to recommend it for certain experimental designs.

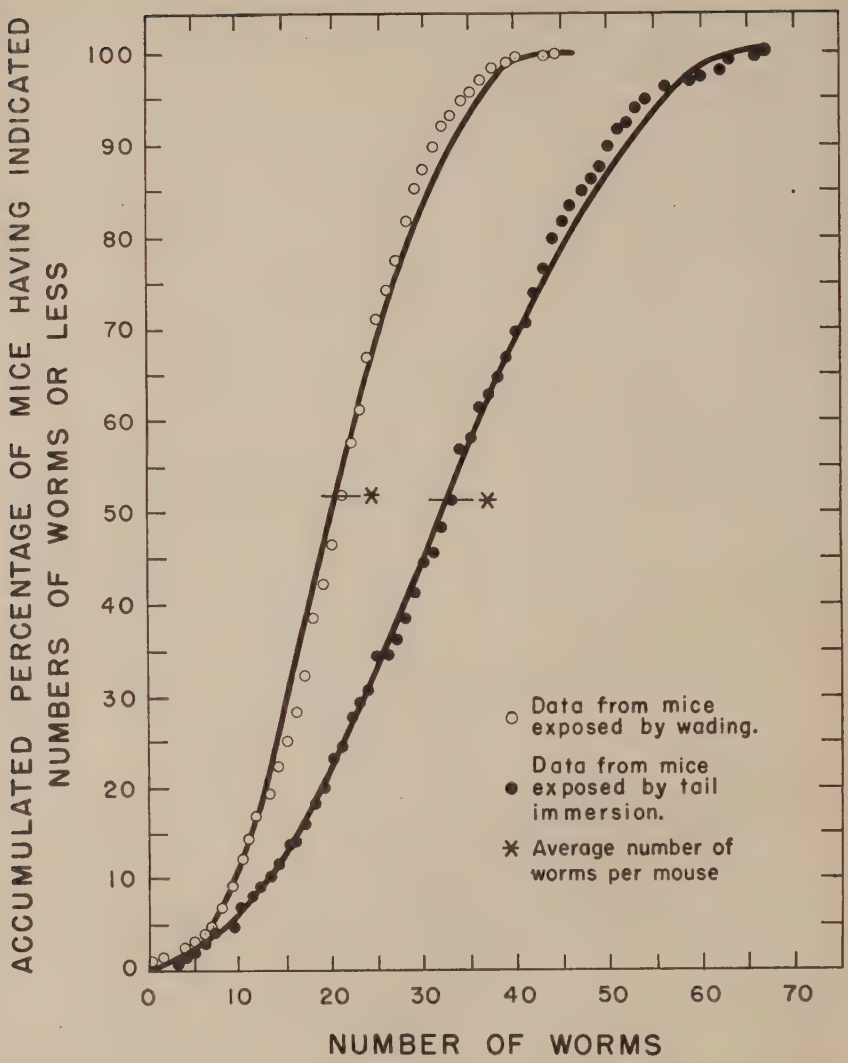

FIG. 2. Accumulative curves (Gompertz) showing worm recoveries from mice exposed to 100 cercariae of *S. mansoni* each by two methods. 523 mice were exposed by letting them wade individually in beakers containing cercarial suspension. Another group comprising 178 mice was exposed by the method described in the present paper. The percentages of worm recoveries for both groups are based on 100 cercariae and do not take into account cercariae recoverable from the beakers or tubes after exposure of the mice.

Contamination of the cercarial suspension by feces and urine with consequent loss of viable cercariae is eliminated. Continuous and optimal host exposure is assured since the mice cannot avoid contact with the cercariae, lick them off the skin, or splash them out of the container. All the cercariae enter the host through a limited area of skin which can be treated or modified under controlled conditions. Biopsy

of the exposed skin area is easy. Finally, all the cercariae entering the host must follow, at least at first, the same path of migration.

SUMMARY

A method of exposing mice to the cercariae of *Schistosoma mansoni* is described which eliminates some of the undesirable features of other procedures, produces generally heavier infections, and permits collection of data not available when other exposure techniques are used.

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SCHISTOSOMA MANSONI AND *S. HAEMATOBIUM* IN THE YEMEN, SOUTHWEST ARABIA: WITH A REPORT OF AN UNUSUAL FACTOR IN THE EPIDEMIOLOGY OF SCHISTOSOMIASIS MANSONI[1]

ROBERT E. KUNTZ[2]

Evidence of the presence of *Schistosoma haematobium* in countries of the Near East, other than Egypt, was first indicated by Sturrock (1899) who reported that schistosomiasis haematobia was widely spread throughout Mesopotamia and as far as 900 miles above the mouth of the Tigris and the Euphrates rivers. Boulenger (1919), Hall (1925) and Neveu-Lemaire (1928) more definitely established the extensiveness of the disease in this part of the world. Felix (1924) discussed the importance of vesical schistosomiasis in Palestine and stated that physicians practicing in that country had known for some time that there was a local center of infection in the environs of Jaffa.

The first real indication that schistosomiasis occurred farther south on the Arabian peninsula was given even before Sturrock's finding of *S. haematobium* in Mesopotamia. Hatch (1887) described twelve cases of vesical schistosomiasis that he had treated in India, where the disease does not occur. His patients were Moslems who had apparently acquired their infections during their pilgrimage to Mecca. Although the Hejaz was assumed to be the site of infection, no information was given concerning other places at which these persons may have contracted schistosomiasis enroute to Arabia. Durand (1926) in reporting schistosomiasis at Djanet in Tunisia also took into consideration the fact that Moslems in the community regularly made the pilgrimage to Mecca and probably returned to Djanet infected with schistosomes. However he realized that they may have acquired their infections on their return trip through Egypt.

Greval (1922), although not in the Yemen, was the first to report schistosomiasis in persons from the Yemen proper. Infected individuals were found among Yemeni admitted to the British hospital in Aden. One Yemeni infantryman from Dhala and a civilian employee from Al-Usmat were infected with *S. haematobium*. Two others, one from Eusafi (? Usaifira), and one from Makatira, while in the Indian Station Hospital, passed ova of *S. mansoni*. Sarnelli (1935), a physician who had resided in San'a, Yemen, for approximately two years, discussed at some length the importance of vesical schistosomiasis in the capital of the Yemen. Although he stated that schistosomiasis of the bladder was prevalent in San'a and vicinity, and found *Bulinus*, he made no mention of *S. haematobium*. Several years later Petrie (1939), also a resident of the Yemen for two years, in a general discussion of the diseases and conditions in the country implied that schistosomiasis was a rather common and an important disease in the Yemen, but he failed to specify the types of disease he had encountered. The prevalence of the schistosomiases in the Yemen,

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The author is indebted to Dr. J. Bequaert, Museum of Comparative Zoölogy, Harvard University, for identification of molluscs taken on the U. S. Naval Medical Mission to the Yemen.

a fact not clearly defined in texts on parasitology or tropical medicine, has attracted the attention of doctors in the Aden Protectorate, as well as in eastern Arabia. These findings are of considerable concern outside of the Yemen inasmuch as large numbers of Yemeni laborers have migrated into the Aden Protectorate, or more recently have obtained employment in the oil fields of the Arabian American Oil Company (Delougherty, 1950).

RECENT FINDINGS

By invitation of the King of the Yemen a small group of investigators from United States Naval Medical Research Unit #3 of Cairo, Egypt, visited the Yemen in Southwest Arabia during January and February of 1951 to make a preliminary medical survey of the Kingdom. The Naval Medical Mission to the Yemen spent approximately seven weeks in the field, its time being rather evenly divided for work at three representative locations: Hodeida, a Red Sea coast city in the Tihama or coastal plain; Ta'izz, the provisional capital of the Yemen situated in the intermediate zone at 4100 feet elevation; and San'a, the country's largest city built on the highland plateau at 7200 feet.

Although time did not allow a detailed medical survey, approximately 500 persons representing a rather wide occupational and age range were examined. The Mission's medical officer performed a physical examination and required each person to contribute urine and fecal specimens, to make a peri-anal swab (NIH type), and to give a blood smear and a blood sample for serological studies. In the meantime other members of the group made biological studies and collections of arthropods, snails and parasites which might bear relation to the diseases recognized in the medical examinations.

At Ta'izz an 'on the spot' examination of all peri-anal swabs showed a surprisingly high incidence of *Schistosoma mansoni* infection and a few persons infected with *S. haematobium*. The anal swab in Arabic countries usually serves as a small fecal sample, as well as a conventional swab since the less efficient stone, clod or stick have not yet been replaced by more refined and hygienic toilet paper. After examining all urine and a limited number of fecal specimens, the finding of both *S. mansoni* and *S. haematobium* inspired an immediate search for snail intermediate hosts.

School children manifesting moderately heavy schistosome infections described several seemingly favorable sites for *Bulinus* in valleys 12 to 15 miles from Ta'izz. Several admitted that they frequently swam and played in small streams and pools. Unfortunately a crowded schedule of work did not permit a visit to these outlying locations. A survey of the Ta'izz area revealed only a limited number of small streams and ground water catchments in which snails might be found. *Biomphalaria boissyi arabica* was found in a small drainage canal about six miles north of Ta'izz and in a small slowly running stream six to eight miles northeast of the city. Although all snails taken at these sites were examined for emerging schistosome cercariae and many were crushed, none were found infected. Several dozens of the same snail dipped from a swimming pool and open water storage basin in the King's garden at Usaifira, one mile north of Ta'izz yielded no schistosomes. Local officials voluntarily gave an account of how a doctor from Ta'izz and several friends had contracted schistosomiasis in the same swimming pool a year or so prior to the Naval Medical Mission's visit to the Yemen.

This preliminary survey indicated that the schistosome infections must have originated at some other source. By deduction it seemed that infected molluscs might be found either in the open storage cisterns common to this country, or in the open ablution basins usually found in the mosques. However, a rather careful examination of garden cisterns and pools in several mosques produced no snails. In spite of these failures, the possibility, if not the probability, of people acquiring infection in mosque basins, where the devout wash almost daily, presented itself so strongly that small boys in Ta'izz were offered a reward for snails. The boys were instructed to search all water catchments in or around mosques. The results were most gratifying, for a few hours later several boys brought a handful of snails to the laboratory.

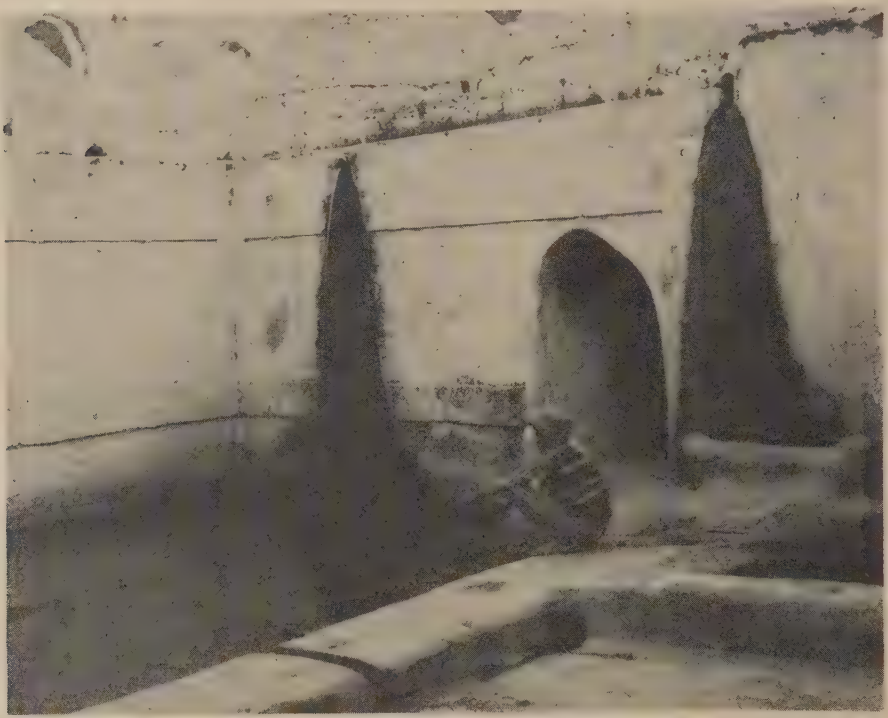

FIG. 1. Open ablution pool in mosque at Ta'izz. The cupolas cover inner individual ablution pools or stalls in which infected *Biomphalaria* were found.

Although the snail collectors would not reveal the exact mosques from which they had obtained negative *Biomphalaria,* several mosques not previously examined by us were visited the following day in company with the Mission's entomologist searching for mosquitoes. The first snail was taken in a mosquito dipper in a small dark, covered ablution room. Further dipping with a snail dip net revealed, that the majority of snails were two or more feet below the water surface clinging to the algae covered walls of a short cemented, stone conduit connecting the inner individual ablution basins with a larger, sunlit basin outside (Fig. 1). The snails were feeding on green and blue-green algae, and on human feces floating semi-buoyantly

in the water. The large basin outside was filled by a small but continuous stream of water originating from an underground spring.

When crushed, approximately 35 per cent of 75 *Biomphalaria boissyi arabica* were found to be heavily infected with *S. mansoni*. A search the following day in another nearby mosque with open ablution basins yielded several more infected snails. Snail shells were found in the drained pools of a third mosque.

At Hodeida, in the lowlands, schistosome ova appeared less frequently in the stools and urines. In questioning the activities of infected persons it was learned that all had recently come from outside the Yemen (particularly Eritrea) or from parts of the country outside Hodeida. No snails were found in the basins in several mosques at Hodeida. In the highlands, at Ma'bar and San'a, even fewer schistosome infections were found, and *S. haematobium* was absent. Although both *Bulinus contortus* and *Biomphalaria* sp. were taken from drainage ditches and from small streams in wadis, none were found infected when crushed.

Bulinus contortus was found in considerable numbers in masses of pondweed (*Potamogeton*) growing in the ancient masonry and open cisterns so common on the San'a plateau. A few *Biomphalaria* sp. shells were taken in cisterns near San'a but no living specimens were found. Living *Bulinus contortus* were collected from an open ablution basin in a mosque at Wadi Dhahr, a small village eight to ten miles to the northwest of San'a. None of the *Bulinus* were infected although the use of the water by man in both the open cisterns and the mosques offered excellent opportunity for completion of the schistosome life cycle.

An absence of *S. haematobium* in San'a and vicinity is rather puzzling if one compares the present report with that of Sarnelli who in 1935 declared that vesical schistosomiasis was very widely spread in San'a and on the adjacent plateau, and that it was considered one of the most important diseases in this area. Although the 1951 survey by the Naval Medical Mission included only 71 persons at San'a and 26 persons at Ma'bar it seems that a few cases of *S. haematobium* would have been detected if the earlier reported disease had been so prevalent.

DISCUSSION

The significance of these findings in the epidemiology of schistosomiasis mansoni is readily appreciated when it is recognized that in Ta'izz with a population of approximately 3,000 persons there are over 20 mosques, each a potential source of infections. The finding of *Bulinus,* though non-infected, in the mosque of Wadi Dhahr indicates a similar potential hazard for transmission of *S. haematobium.* Each mosque is visited daily by dozens of persons. Children become infected at a rather early age since males begin to fulfill their religious obligations at the age of eight or nine years. The schistosome hazard is not restricted to the male sex entirely since some cities, such as Ta'izz, have provided a mosque for women.

Schistosomiasis until this time has been considered more or less an occupational or domestic disease. In China, Japan and in the Philippine Islands infection with *S. japonicum* is usually acquired by laborers in rice nurseries, rice paddies and associated irrigation systems, by persons washing clothing and household utensils in infected waters, or occasionally by bathers. Much of the population of Venezuela and certain of the Caribbean Islands are similarly infected in irrigation canals and the disease is thus associated with agriculture. In Egypt, schistosomiasis is essentially an occupational disease among the agricultural workers of the fellaheen. Al-

though the Egyptian fellaheen lives in intimate association with irrigation waters and often performs his ablutions in these waters, his religious obligations do not bring him into contact with such a highly efficient life cycle potential and hazard as reported here for the Yemen. It seems, however, that a similar danger may have existed in the mosques of Egypt before the Ministry of Health outlawed stagnant and misused ablution pools, and demanded that all water for such purposes be piped in from the city's water supply.

Factors governing the effective continuation of the life cycle of *S. mansoni* and possibly of *S. haematobium* in the Yemeni mosques are unique. The control of schistosomiasis mansoni in mosques should be a relatively simple procedure. Periodic drainage and scrubbing down of walls of the basins would reduce not only the schistosome hazard but also serve as a break in the transmission cycle of water borne enteric diseases. This method could be executed with only moderate effort since many of the mosques, especially those in Ta'izz, are situated on slopes which would allow for easy drainage. The second choice would be treatment of the ablution basins of all mosques twice a year with a suitable molluscacide. Although drainage of basins is the cheaper of the two methods suggested for destroying snail hosts it was estimated that all mosques in Ta'izz could be treated twice yearly with dinitro-o-cyclohexylphenol for forty dollars or less.

SUMMARY AND CONCLUSIONS

1. The first medical survey of the Yemen, S. W. Arabia has confirmed earlier, rather indefinite, reports that both *Schistosoma mansoni* and *S. haematobium* occur in the Yemen.

2. *S. mansoni* is very common at Ta'izz, the provisional capital of the Yemen located at 4100 feet, but is of less concern at Hodeida on the Red Sea coast or at San'a the Yemen's largest city, on the highland plateau at 7200 feet.

3. *S. haematobium* is found principally at Ta'izz. Numerous cases of infection by the latter schistosome originate in Eritrea.

4. Observations made on limited numbers of persons during January and February of 1951 in the Yemen indicate that the majority of cases of schistosomiasis mansoni are probably contracted in the ablution basins of mosques. This is the first report showing a clearcut relationship between religious practices and schistosome infection. Such a pattern is unique in the epidemiology of schistosomiasis.

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HUMAN SPARGANOSIS IN SOUTH TEXAS

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The larval cestode, *Sparganum mansoni,* has been reported frequently as a human parasite in the Orient. On the other hand, reported cases of human sparganosis from North America are few. The first U.S. case of human infection with a pseudophyllidean larva was reported from Florida by Stiles (1908), and the worm was identified as *Sparganum proliferum.* Stiles' case is apparently the only North American record for this form although, in the same paper, Stiles mentioned another supposed but unconfirmed case of *S. proliferum* in man. The second unquestionable human case of sparganosis was reported by Moore (1914) from Texas. The worm was identified by Dr. B. H. Ransom as *Sparganum mansoni.* Additional natural infections in man in the U.S. have not been reported.

Mueller and Coulston (1941) established successful experimental infections in man with *Spirometra mansonoides* (Mueller, 1935). These workers, using themselves as experimental subjects, reported extensive experiments in which a sparganum, after human passage, was fed to a cat, and the adult worm recovered. Procercoids from this worm were then used in producing sparganosis in monkeys. Valuable data concerning the immune response in man were recorded.

A second case of human sparganosis in South Texas has recently come to the attention of the writer. Appreciation is expressed to Drs. H. H. Duke of Baytown, Texas, and Violet Kieller of Hermann Hospital, Houston, who, as physician and pathologist respectively, made the present material available and furnished valuable data in connection with this case.

This patient, a woman of 42 years, was first seen at Baytown, Texas, on July 27, 1950, when she visited her physician complaining of a mass in her right breast. Upon examination, head, neck, chest, heart, and abdomen were normal. In the right breast, about one inch above and one inch lateral to the nipple, there was observed a discrete nodule about the size of a large pea. The nodule was painless and showed no surface inflammation. It was apparently in the subcutaneous fat and was freely movable. A tentative diagnosis of a sebaceous cyst was made, and the patient was instructed to return if it increased in size, showed signs of inflammation, or became painful.

On August 18, 1950, the patient returned to the physician complaining of pain in the nodule. Examination revealed a circular area of inflammation about 1½ inches in diameter at the site of the lesion. Immediately above the lesion there was a dark blue area. It was suspected that the tissues had been subjected to manual pressure or squeezing and that a subcutaneous hemorrhage had resulted. The patient denied such manipulation. At this time the patient showed a normal total white and red blood cell count, but a differential white cell study showed an eosinophilia of 9%.

At this time the mass was surgically removed under local anaesthesia. The nodule lay in the subcutaneous fat. At the time of removal the surgeon noted a fine white filamentous object protruding from a break in the nodule. Since this thread-

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like object showed movement, the surgeon carefully preserved the material for further examination. Study of the object removed from the nodule showed that it was a tapeworm larva of the sparganum type. The larva was 7.5 cm. long and about .05 cm. in diameter and possessed a well developed scolex similar to that of members of the genus *Spirometra*. Unfortunately, the larva was dead when received by the writer and feeding experiments were not possible.

The tumor measured $3 \times 2 \times 1.5$ cm., had a yellowish-white smooth surface, and was sharply circumscribed in the surrounding fat. It was not encapsulated. Study of sections revealed that the tissue involved was fatty with some fibrous elements. No glandular tissue was found. Surrounding the parasite, there was a marked inflammatory infiltration, primarily of lymphocytes. Neutrophiles were few, and giant cells were not observed. Eosinophiles were quite numerous in some areas. There was no evidence of neoplastic tissue.

The patient was dismissed with an admonition to examine herself daily for the occurrence of additional lesions of this type. On September 18, exactly a month later, the patient returned to the physician to complain of a nodule in the middle of the medial side of the left thigh. At this time the toal white and red blood cell counts were normal; the patient showed an eosinophilia of 4%. The mass was removed at once by the same procedure as the previous lesion.

According to the surgeon and the pathologist who examined this second specimen, it was quite similar to the previous one. The histological picture was reported to be precisely the same. This second specimen was inadvertently destroyed before the writer had an opportunity to examine it, but, since the surgeon and pathologist had previously examined the worm and lesion from the breast, it seems reasonable to accept their opinion that the two lesions were identical.

Following the removal of the second sparganum and to the present date, no additional worms have been recovered from this patient. On June 20, 1951, a study of the blood showed an eosinophile count of 1%.

An attempt was made to obtain information from the patient which would allow intelligent speculation as to the mode of infection. The patient stated that she did not fish, hunt, nor swim. She had not eaten wild game in several years nor been in conscious proximity to any reptiles or amphibians. The sole source of drinking water, insofar as she could remember, had been the municipal water system. She further stated that she did not own nor come in contact with cats, although the family owns a small dog, a spaniel. She has worked for some time in the office of a dry cleaning-laundry establishment. The foregoing information gives no real hint as to how this individual became infected, but it seems most likely to the writer that the worms gained entrance *per orum*. It seems probable that she may have partaken of shallow well water and might not recall such an incident rather than that she would have forgotten a swimming episode. This speculation is of course based on the supposition that infection was contracted by contact with or swallowing of the arthropod intermediate host, a copepod.

It is of interest to note that Moore's case (1914) also occurred as a lesion of the breast. It is impossible to say whether this is mere coincidence, because lesions of the breast are more likely to result in consultation with a physician, or whether the worm shows some predilection for this site. Moore's case had a history of having lived in Florida some eight years before, and it was not clear as to whether Moore's

case bore any relation to the case previously reported from Florida by Stiles (1908). In the present case there is little doubt, since the patient is native-born and little-traveled, that the worms were obtained in this area. It seems highly probable that a relationship exists between this and Moore's case of sparganosis in South Texas and the finding of *Spirometra mansonoides* in feral domestic cats and in wild cats, presumably *Lynx rufus,* from the same area (Read, 1948). Mueller and Coulston (1941) pointed out that human sparganosis may be much more common in the U.S. than previous records would indicate and that all "cysts" and "fatty tumors" removed from under the skin should be suspect. These workers remarked ". . . it seems therefore highly probable that when physicians throughout this region become educated to look for the worm and to recognize the symptoms of sparganum infection, many more cases will be found and reported," an opinion with which the present writer thoroughly agrees. The detection of the case reported in this paper was due entirely to the alertness of the physician.

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STUDIES ON THE HELMINTH FAUNA OF ALASKA. IX—THE CESTODE PARASITES OF THE WHITE-FRONTED GOOSE *(ANSER ALBIFRONS)* WITH THE DESCRIPTION OF *HYMENOLEPIS BARROWENSIS* N. SP.

EVERETT L. SCHILLER*

Although the breeding range of the white-fronted goose, *Anser albifrons* (Scopoli), is practically circumpolar and this goose is common throughout most of the Northern Hemisphere, the literature reveals little knowledge of its parasites.

During the continuation of helminthological studies at Point Barrow, Alaska, in the spring of 1951, 35 white-fronted geese, including 16 males and 19 females, were taken on the Arctic Coast breeding grounds. For the most part these were collected in early June along the Inaru river, near the delta of the Meade, about 50 miles south of Barrow Village. Twelve birds (34%) were found parasitized by cestodes of the genus *Hymenolepis* Weinland, 1858. Four of the geese contained *H. creplini* (Krabbe, 1869), which has been recorded previously from this host. Eight harbored a species of *Hymenolepis* which appears to be unknown and therefore is herein described as new. These two species of cestodes were not found to occur simultaneously in the same individual host.

Two other white-fronted geese were collected and examined at Anaktuvuk Pass, Brooks Range, Alaska, in May, 1950, by Dr. Robert Rausch of this laboratory. Both were negative.

The writer wishes to take this opportunity to express his appreciation to Dr. Ira L. Wiggins, Director, Arctic Research Laboratory, Office of Naval Research at Point Barrow, Alaska, and his staff, for providing laboratory space and autopsy facilities and for their excellent cooperation in connection with this work.

Hymenolepis barrowensis n. sp.
(Figs. 1–5)

Diagnosis: Length of strobila about 70 mm.; maximum width 1.5 mm., attained at posterior end. Scolex 72×96 microns. Suckers, about 40 microns in diameter, weakly muscled and unarmed. Invaginated rostellum 84 microns in length and 35 microns in diameter at base. Rostellum provided with single row of 10 hooks 22 microns in length. Strobila 19 microns wide immediately posterior to base of scolex. Genital pores unilateral and dextral. Genital ducts pass between dorsal and ventral excretory canals. Cirrus sac averages 320 microns in length by 56 microns in width. External seminal vesicle slender, 288 microns long by 52 microns in diameter. Cirrus heavily armed with recurved spines 8 to 10 microns in length. Sacculus accessorius and cirrus stylet absent. Testes three in number, subspherical, about 129×144 microns in mature proglottids. Testes usually lie in same plane in straight line between excretory canals. Ovary deeply lobed. Ovary and vitelline gland located aporal to midline of proglottid. Poral

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EXPLANATION OF PLATE

Morphological details of *Hymenolepis barrowensis* n. sp.

FIG. 1—Scolex.
FIG. 2—Rostellar hook.
FIG. 3—Spines of cirrus.
FIG. 4—Late mature proglottid (ventral view).
FIG. 5—Pre-gravid segments showing early development of uterus.

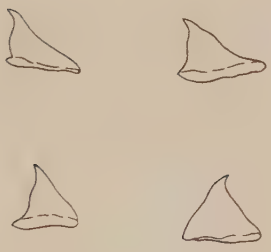
PLATE I

50 μ

1

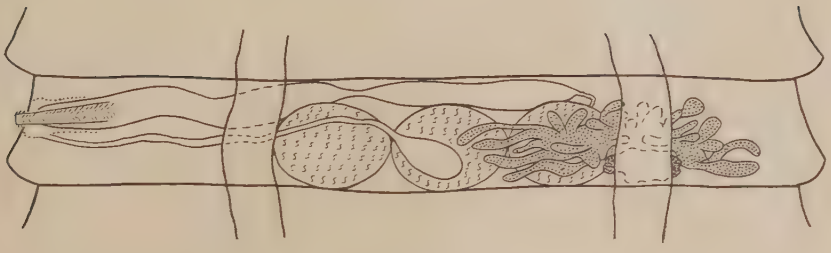
10 μ

2



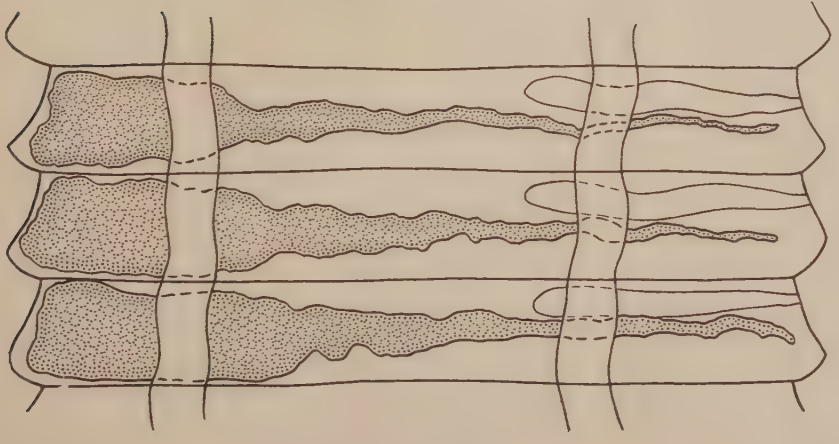
10 μ

3



500 μ

4



500 μ

5

lobes of ovary completely overlap aporal testis and extend to midline of middle testis in mature proglottids. Aporal lobes of ovary extend beyond aporal excretory canals nearly to margin of proglottid. Vitelline gland irregular in shape, about 72 microns in diameter. Vagina posterior and ventral to cirrus sac, 16 microns in diameter. Uterus extends transversely across proglottid as a slender irregular tube and passes dorsal to and extends beyond excretory canals. Uterine growth considerably more rapid in aporal one-fourth during early development. Completely gravid segments not observed. Ventral longitudinal excretory canals measure 43 microns in diameter; dorsal canals, 14 microns in diameter.

Host: Anser albifrons (Scopoli).

Locality: Inaru River, near Point Barrow, Arctic Coast, Alaska.

Habitat: Small intestine.

Type: One slide, No. 37341, containing an entire specimen, has been deposited in the Helminthological Collection of the U. S. National Museum.

Discussion: Of the avian species of *Hymenolepis* possessing 10 rostellar hooks, a spinose cirrus, an ovary and vitelline gland nearly aporal to all testes and lacking a cirrus stylet as well as a sacculus accessorius, the new species is most closely comparable to *H. setigera, H. bilateralis* and *H. ardeae.* These were further considered for comparison and found to differ from *H. barrowensis* n. sp. as follows:

H. setigera (Frölich, 1789) (parasitic in Anseriformes) has a strobila of greater length (200 mm.), a bilobed ovary and a vagina which has a sphincter. The rostellar hooks are somewhat similar in shape but are of considerably greater length (35–44 microns).

H. bilateralis von Linstow, 1905 (parasitic in Anseriformes) has longer rostellar hooks (33 microns) of a distinctly different shape.

H. ardeae Fuhrmann, 1906 (parasitic in Ardeiformes) has a strobila of greater length and width (125×3 mm.), a shorter cirrus sac (280 microns) and rostellar hooks of much greater length (45 microns) of a quite different shape.

The differential growth rate of the aporal one-fourth of the uterus appears to be a well-marked character in the specimens of *H. barrowensis* available for this study; however, until more material is examined to determine the consistency of this character for purposes of diagnosis, it must remain one of value only in facilitating recognition of the species.

The morphological characters exhibited by *H. barrowensis* support further the opinion of the writer (1951) concerning the nonvalidity of *Drepanidotaenia* as a subgenus. The new species is another which might well be added to the ever-growing number belonging to the genus *Hymenolepis* which possess other than eight hooks in combination with characters (testes in a transverse row and an ovary and vitelline gland aporal to the testes) previously considered to be significant relative to the concept of *Drepanidotaenia* as a genus or subgenus.

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EFFECTS OF THE NEMATODE *OESOPHAGOSTOMUM VENULOSUM* ON SHEEP AND GOATS

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INTRODUCTION

Three species of *Oesophagostomum* occur in domestic ruminants. Two of these, *O. columbianum,* in sheep and goats, and *O. radiatum,* in cattle, have been experimentally investigated by Veglia (1928), Fourie (1936), Andrews and Maldonado (1942), Sarles (1944), and Mayhew (1948), and were found to have rather serious effects on these animals. The primary host reaction occurs in the digestive tract, owing to the protracted stay and migration of the larvae in the wall of the intestine. There has been little experimental investigation of the effects of the third species, *O. venulosum,* which is widespread and occurs frequently in sheep and goats. The purpose of the present study was to determine the effects of this species on the host animals under various experimental conditions.

MATERIALS AND METHODS

All animals, 29 Shropshire × Hampshire sheep and 3 kids, used in this study were free of helminth parasites except for a few *Strongyloides.* The animals varied in age from 2 to 18 months at the time of initial infection. They were maintained under conditions designed to prevent extraneous infection, except for 3 animals which were allowed to pick up natural infections of *O. venulosum* by being kept in the same pens with infected animals. Rarely a few *Nematodirus,* never exceeding a dozen worms, usually with an egg count of 2 and not exceeding 10 per gram of feces would be acquired by an experimental animal. These small numbers of worms were considered to be of no consequence in this study.

Eggs of *O. venulosum* from 32 females were cultured, and the resulting infective larvae administered to a lamb. A month later the eggs of this species were present in the feces. The feces of this lamb and that of others infected through it were the source of culture material. The experimental animals were infected with larvae from 7 to 70-day-old cultures. The number of larvae administered to a host animal varied from 20 to 28,000. For each inoculation the larvae, suspended in 10 to 15 ml. of water, were administered by pipetting them down the throat of the animal. Some animals were given single inoculations, and others were given several inoculations over a period of time. After the termination of the initial infections, 14 animals were reinfected, and one of these was reinfected a second and third time. Altogether, 48 infections were studied. The course of each infection was followed by means of egg counts. The effects on the health of the animals were noted. The animals were necropsied 3 to 694 days after infection, the worms recovered, and the digestive tract and other tissues thoroughly examined for lesions. When more than 1,000 worms were recovered at necropsy, dilution counts of the worms were made and the total number computed. All other counts were actual counts of the total number of worms recovered.

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EFFECTS OF INFECTION BY SINGLE INOCULATION

A summary of the circumstances of infection by single inoculation up to the time of development of mature adult worms in approximately one month, and of the pathologic conditions noted at postmortem, is presented in table 1. On the third day after infection the larvae were encysted in the mucosa of the small intestine. On subsequent days they were free in the lumen. By the fifth day after infection 99 per cent of the worms recovered had already migrated to the cecum and remainder of the large intestine, the locations of mature adult worms. Fifteen days after infection many young adult worms were passing out with the feces. The greatest number of ulcerative lesions at postmortem were noted on the 13th and 15th days after infection. They did not exceed 200 in number and 2 mm. in diameter, and they did not contain larvae nor coccidia. A few petechiae 1 mm. in diameter were noted in the cecum on the 30th day after infection.

Sixteen animals were necropsied more than a month after infection. The circumstances of each infection, symptoms noted during infection, and the pathologic conditions noted at postmortem are presented in tables 2 to 4. Four animals (table 2) were never reinfected and 12 were reinfected after the termination of the initial infection, 8 (table 3) by another single inoculation, 1 (table 3) three times by single inoculations after the termination of each previous infection, and 3 (table 4) by exposure to natural infection with *O. venulosum* in a pen containing infected animals. Beginning about 15 days to one month after infection 14 of the 16 animals showed one or more of the following symptoms: soft to diarrheic stools, and pellets covered with excessive mucus occasionally specked and streaked with fresh blood. Of the 12 reinfected animals 6 showed these symptoms only in the initial infection, 2 only in the second infection, 2 in both infections, and 2 in neither infection. The symptoms lasted for variable periods of time, frequently approximately half a month, usually with a gradual return to normal.

In general the egg output increased as the number of larvae administered increased up to approximately 1,500. Thereafter, it usually decreased as the number of larvae administered increased. However there was considerable variation in the egg output even between animals fed the same number of larvae. The longest egg laying period was approximately 400 days.

EFFECTS OF MULTIPLE INFECTION OVER A PERIOD OF TIME

Nine lambs, 3½ months old when first inoculated, were divided into 3 comparable groups of 3 lambs each, sex, weight, rate of gain in weight, and age being taken into account in making up the groups. The groups were kept in contiguous, similar pens, and were maintained under conditions designed to prevent extraneous infection with helminths. Each group was fed the same amount of alfalfa hay and mixed grain.

One group was used as a control. A second group was fed a moderate number of *O. venulosum* larvae, the total number being 4,370 larvae per lamb given in 6 inoculations during 23 days. The third group was fed a greater number of larvae, a total of 28,000 per lamb being given in 31 inoculations during 94 days. The control group was given 20 doses, each of 10 ml. of dilute culture medium fluid only, over a period of a month, to test it for possible pathogenic effect.

Weight changes: The lambs were weighed at weekly intervals. Four days be-

TABLE 1.—*Data on lambs infected by single inoculations with infective larvae of* Oesophagostomum venulosum *and necropsied during larval stages and up to the time of development of mature adult worms*

Lamb No.	Age at time of infection (months)	Age of larval culture (days)	Larvae fed	Interval between infection and necropsy (days)	Worms recovered at necropsy	Per cent in each stage						Pathologic conditions noted at postmortem
						3rd	E.[1] or M.	4th	E.[1] or M.	Young adult	Mature adult	
999	7	19	10,000	3	3,836	100						90% of larvae encysted in wall of small intestine
982	6	7	10,000	4	159	4	20	76				None
906-1	3.5	14	5,000	5	1,888		2	98				None
992	3	34	250	11	47			100				None
A 14	11	29	2,200	13	1,139			99.56	0.09	0.35		App. 200 yellowish, ulcerative lesions 1–1½ mm. in diameter in small intestine. No larvae nor coccidia in them.
904-1	5	7	7,000	15	3,524			10	6	84		App. 200 ulcerative lesions 1–2 mm. in diameter in small intestine, some with necrotic tissue in center but no larvae nor coccidia. Excessive mucus on pellets.
975	3	34	250	21	45					100		None
A 15	7.6	27	1,400	24	400					97	3	Pellet formation delayed, pellets not formed until some distance beyond the crown.
A 61	10	29	500	30	457			3		66	31	Few petechiae 1 mm. in diameter in cecum.
A 37	5	28	1,500	31	400					0.5	99.5	None

[1] Ensheathed or moulting.

TABLE 2.—*Data on sheep infected once with a single dose of* O. venulosum *larvae and necropsied more than a month after infection.*

Sheep No.	Age at time of infection (months)	Age of larval culture (days)	Larvae fed	Peak egg count (per gram of feces)	Patent period (days)	Interval between infection and necropsy (days)	Worms[1] recovered at necropsy	Symptoms noted during infection	Pathologic conditions noted at postmortem
993	18	28	1,500	552	57	110	6	Diarrhea; mucus specked with blood	Ten 1–1½ mm. nodules, with masses of hard material, in small intestine
A 67	11.4	70	500	1,176	259+[2]	289	111	Excessive mucus on feces	Enterotoxaemia, *Clostridium* sp. suspected
A 62	11.4	70	500	34	[3]	323	33	Stool soft, pellets obliterated	None
911	13.6	10	1,500	396	142	629	4[4]	Feces formed but not in pellets; mucus specked with blood	None

[1] All worms were mature adults, unless otherwise noted.
[2] Died still patent.
[3] Necropsied before end of patent period.
[4] 2—4th stage larvae, 2 mature adults, perhaps due to natural reinfection.

TABLE 3.—*Data on sheep and goats infected by a single dose of* O. venulosum *larvae, and reinfected by a single dose, after the termination of the initial infection. One sheep was reinfected a second and third time*

Sheep or goat (G) No.	Age at time of infection (months)	Age of larval culture (days)	Larvae fed	Peak egg count (per gram of feces)	Patent period (days)	Interval between infection and necropsy (days)	Worms[1] recovered at necropsy	Symptoms noted during infection	Pathologic conditions noted at postmortem
G-777	5	34	1,000	1,380	115	208	525[3]	None	None
	11	36	4,500	....	[2]	39		Fresh blood on pellet	
908	2	19	20	182	78	379	7	None	None
	6	32	700	388	110	245		None	
	10.5	16	675	1	1–7	117		None	
	12.5	12	1,000	14	[2]	58		None	
A 12	8	29	900	310	78	213	None	Diarrhea	Died of enterotoxaemia. *Clostridium* sp. suspected
	12.5	70	500	0	0	68		None	
A 1	4.5	10	4,200	84	1–7	278	None	Feces formed but not pellets	Three 1–1½ mm. nodules, containing masses of brownish material, in small intestine
	10	29	1,500	0	0	111		Pellets soft, almost obliterated	
940	13	10	2,000	84	105	291	None	Feces formed but not pellets	Excessive mucus in large intestine and cecum
	19	29	1,500	0	0	125		None	
941	15	24	500	260	115	556	30[4]	Feces formed but not pellets; fresh blood on pellets	None
	23.5	70	500	28	[2]	307		Excessive mucus; blood specks; diarrhea	
968	8	15	4,000	100	71	766	5[5]	Diarrhea; thick excessive mucus specked with blood	Excessive mucus in large intestine
	18	28	1,500	66	119	446		None	
907	4	22	350	1,504	135	694	None	None	None
	10.5	14	500	848	377	484		None	
959	3	22	350	4,652	144	689	2	None	One 2 mm. calcareous nodule, containing debris, in cecum
	10	11	1,500[6]	992	400	489		Feces formed but not pellets	
		14	3,000						

[1] All mature adults unless otherwise noted.
[2] Necropsied before end of patent period.
[3] 473—4th stage larvae, 33 young adults, 19 mature adults.
[4] Suspect natural reinfection.
[5] One 4th stage larva, 4 mature adults. Suspect natural reinfection.
[6] Two inoculations, 21 day interval.

TABLE 4.—*Data on lamb and kids infected once by a single dose of* O. venulosum *larvae, and once with* O. venulosum *by natural means in their pens, after the termination of the first infection*

Lamb (L) or kid (K) No.	Age at time of infection (months)	Age of larval culture (days)	Larvae fed	Peak egg count (per gram of feces)	Patent period (days)	Interval between infection and necropsy (days)	Worms[1] recovered at necropsy	Symptoms noted during infection	Pathologic conditions noted at postmortem
L-989	App. 8	..	Natural infection	592	App. 130	App. 433	4	Diarrhea	Three 1½ mm. nodules with masses of hard material, in cecum
	18	28	1,500	6	1–7	114		None	
K-778	5.5	47	2,000	200	108	302		Diarrhea	
	App. 10	..	Natural infection	380	[2]	App. 161	21	None	None
K-740	6	34	5,000	140	131	489		Diarrhea	
	App. 11	..	Natural infection	160	App. 272	App. 335	1	None	None

[1] All worms were mature adults.
[2] Necropsied before end of patent period.

fore the moderate-dose group was first inoculated, it had the same weight as the control group. The greatest difference in weight in favor of the control group over the moderate-dose group occurred 66 days after the initial inoculation. At this time the moderate-dose group weighed 95.9 per cent as much as the control group. The control group maintained its slight advantage in weight for approximately 2 months after the final inoculation. From the second to the fourth month after the final inoculation, the moderate-dose group weighed slightly more than the control group.

Five days before the heavy-dose group was first inoculated, it weighed 96.9 per cent as much as the control group. The greatest difference in weight in favor of the control group occurred 86 days after the initial inoculation and 8 days before the final inoculation. At this time the heavy-dose group weighed 92.5 per cent as much as the control group. Two months after the final inoculation the heavy-dose group weighed 95.4 per cent as much as the control group.

Blood examinations: Five times during the experiment, at approximately monthly intervals, hematocrit readings were taken on blood obtained from the jugular vein of each sheep. The data are presented in table 5. The first of the

TABLE 5.—*Data on blood examinations of controls and animals infected with* Oesophagostomum venulosum

Date	Per cent packed blood cells								
	Control lambs			Moderate-dose lambs			Heavy-dose lambs		
	A14	A57	A61	A16	A17	A56	A18	A21	A60
6-3-49[1]	35	37	35	41	38	37	35	37	36
7-6-49	39	31	31	35	36	34	33	35	35
8-22-49	43	37	34	40	41	42	37	37	38
9-29-49	41	37	35	41	44	36	34	37	39
11-21-49	45	40	34	39	43	38	40	42	42

[1] This examination was made 10 days prior to infection of the moderate-dose group and 4 days prior to infection of the heavy-dose group. All other examinations were made during and after infection.

five sets of readings was taken before the initial inoculations. The others were taken during and after the infections. In all cases the blood was centrifuged at 2,300 r.p.m. for 50 minutes. No significant change between groups in the per cent packed blood cells was noticed during the experiment.

Clinical evidence of infection: The condition of the stool was considered to be a clinical indication of the effect of the infections. During the experiment several indications of intestinal irritation were noticed in the feces of the moderate-dose and heavy-dose groups but not in the feces of the control group. In the moderate-dose group soft non-pellet masses were first passed on the 15th day after the first inoculation. Twenty-one days later, the feces had the consistency of soft cow manure. About a month later, when pellets were again formed, they were covered by excessive mucus, occasionally containing streaks and specks of fresh blood. Slightly flattened pellets, clumped by mucus, were passed for an additional month. Thereafter normal, rounded, free pellets were passed.

In the heavy-dose group soft, non-pellet masses were first passed on the 17th day after the initial inoculation. Twenty-five days later, the feces had the consistency of soft cow manure. For approximately 69 days thereafter, about 17 days after the final inoculation, symptoms of intestinal irritation were evident. During

this period pellets were passed again, but they were usually clumped and covered by excessive mucus, occasionally containing specks and streaks of fresh blood. Thereafter the feces remained normal.

To obtain an indication of the relative number of mature adult worms remaining in the intestine, weekly egg counts were made. In preliminary work carried out under circumstances similar to those in the present experiment, light doses of larvae, an average of 1,817 per animal, administered in a single inoculation to 4 lambs and 3 kids, resulted in an average peak egg output of 791 eggs per gram of feces 60 days after infection. In the lamb with the longest patent period, the feces became free of eggs approximately 170 days after infection; the average was approximately 140 days. In the present experiment the moderate-dose group reached a peak output of 289 eggs per gram of feces 36 days after the initial inoculation; in the lamb with the longest patent period, the feces became free of eggs approximately 145 days after the initial inoculation. The average was approximately 130 days. The heavy-dose group reached a peak output of 51 eggs per gram of feces 38 days after the initial inoculation. In the next 24 days the egg count dropped to an average of 3 per gram, and became zero approximately 80 days after the initial inoculation. The egg count data in these three groups are presented in figure 1.

Reinfection after termination of initial infection: One hundred eighty-two and 117 days after the final dose of larvae had been fed to the moderate-dose and heavy-dose groups respectively, and after the initial infections in all lambs had terminated, one lamb from each group, including the control group, was inoculated at the same time with 500 larvae from the same batch. Lamb A61, formerly in the control group, was necropsied 30 days after infection, and 457 *O. venulosum,* 14 fourth stage larvae, 301 young adults, and 142 mature adults were recovered. Lamb A16, in the moderate-dose group, was necropsied 28 days after infection, and 31 worms, 8 fourth stage larvae, 8 young adults, and 15 mature adults were recovered. Lamb A18, in the heavy-dose group, was necropsied 26 days after infection, and one mature adult and no larvae were recovered.

Postmortem observations: One of the other two lambs in the moderate-dose group was necropsied 5 months after its final inoculation. Three adults were recovered from the cecum. The last lamb in the moderate-dose group was necropsied $5\frac{1}{2}$ months after its final inoculation and no worms were recovered. One of the other two lambs in the heavy-dose group was necropsied 2-2/3 months after its final inoculation. Two adults were recovered from the cecum. The last lamb in the heavy-dose group was necropsied $3\frac{1}{4}$ months after its final inoculation. Two adults were recovered from the cecum.

There were no serious permanent effects on the tissues. At necropsy all the lambs had an abundance of fat stored throughout the body, and they were in good condition. Minor adhesions were present in some of the lambs which had been fed larvae. The digestive tract from the beginning of the abomasum to the rectum was thoroughly examined, but no nodular lesions larger than 2 mm. were found, and these were rare. No lesions were found in the other organs. Since the experimental lambs showed no evidence of significant permanent pathologic conditions, and the fecal examinations of the controls had always been negative, it was deemed unnecessary to necropsy the 2 remaining control lambs. A summary of the circumstances of the infections in this experiment and the data obtained are presented in table 6

TABLE 6.—*Data on lambs fed several doses of* Oesophagostomum venulosum *larvae over a period of time*

Lamb No.	Age at time of infection (months)	Average age of larval culture (days)	Period over which larvae were fed (days)	No. of doses	Total larvae fed	Peak egg count (per gram of feces)	Patent period (days)	Interval between infection and necropsy (days)[1]	Worms[2] recovered at necropsy	Symptoms noted during infection	Pathologic conditions noted at postmortem
A 17	3.8	21	23	6	4,000	292	99	169	None	Diarrhea; mucus specked with blood	None
A 56	3.25	21	23	6	4,700	288	119	147	3	Diarrhea	One nodule in small intestine; minor adhesions of liver
A 16	3.8	21	23	6	4,400	535	100	187	31[3]	Diarrhea; excessive mucus, specked with blood	Minor adhesions of liver
	10.6	29	1	1	500	..	..	28		None	
A 61[4]	10	29	1	1	500	..	..	30	457[5]	None	Few petechiae 1 mm. in diameter in cecum
A 18	3.4	21	94	31	28,000	2	1–7	143		Diarrhea; excessive mucus, specked with blood	Minor adhesions of cecum; few 1 mm. lesions in small intestine
	10.5	29	1	1	500	..	..	26	1	None	
A 21	3.4	21	94	31	28,000	140	44	97	2	Diarrhea; excessive mucus; blood on pellets	None
A 60	3	21	94	31	28,000	44	44	80	2	Diarrhea; blood-specked mucus	None

[1] Days after final dose.
[2] All mature adults unless otherwise noted.
[3] 8—4th stage larvae, 8 young adults, 15 mature adults.
[4] Control in reinfection of A 16 and A 18.
[5] 14—4th stage larvae, 301 young adults, 142 mature adults.

DISCUSSION

The number of mature adults becoming established in the intestine could not be increased indefinitely by increasing the number of larvae administered in a single dose or by feeding a great number of larvae over a long period of time. As indicated by the egg output and the length of the patent period, the greatest number of mature adults became established in the intestine when 350 to 1,500 larvae were administered in a single dose, and the least when many larvae, as many as 28,000, were administered over a long period. For example, compare the histories of lambs 907, 959, and kid G-777 (table 3) with those of lambs A18, A21, A60 (table 6), A1, 968 (table 3), and kid K-740 (table 4). Increased feeding and extended feeding of larvae merely resulted in more worms being scoured out, probably due to greater irritation of the intestinal mucosa. Also, in natural infections acquired on infested pastures, mature, adult *O. venulosum* were not found in great numbers at necropsy.

The symptoms of infection noted occurred only during the adult stage of the worms and may have been caused by their feeding on the intestinal mucosa. The adult buccal capsule appears to be well adapted for this use. There are 18 lip-like structures in the external corona radiata surrounding the mouth and 32 sclerized denticles in the internal corona. The worms are not attached to the mucosa as are blood-sucking worms. Instead, at necropsy, they are found moving about in the feces or adjacent to the intestinal wall. The petechiae occasionally noted in the cecum at postmortem, and the specks and streaks of blood in the excessive mucus resulting from the irritation to the intestine, are believed to be incidental to the worms' feeding on the epithelium. The freshness of the blood indicates its source from the cecum and remainder of the large intestine, the locations of mature *O. venulosum*. Some or all of these symptoms were noted in cases of infection by single inoculation, by multiple inoculation, and in animals naturally infected from others in the same pen. The symptoms can be continued for at least 3 months by repeated dosing with larvae for that period. The digestive disturbance had a slight detrimental effect on the weight gain of the animals.

The lesions noted at postmortem were most abundant approximately half a month after infection. They were ulcerative lesions 1 to 2 mm. in diameter, approximately 200 in number, and located in the small intestine. They may have been points where larvae had emerged from the mucosa or points of temporary attachment of the worms. A total of approximately 180,000 larvae had been fed to the 32 experimental animals. However, nodules in the intestine were very rare at postmortem, only one animal having as many as 10, and these did not exceed 2 mm. in diameter. No larvae were found in any of the nodules. If the nodules were due to retention of larvae in the mucosa, it was the very rare exception to the usual course of events. Ordinarily, the larvae returned to the lumen from the intestine within one day after they entered the mucosa and they did not result in marked local reactions. Under conditions similar to those of this study, feeding 180,000 larvae of *Oesophagostomum columbianum* to 32 sheep or goats would result in several thousand nodules, and many of these would be as much as 5 mm. in diameter. Approximately 25 larvae removed from nodules in the small intestine, cecum, and remainder of the large intestine of 3 sheep and 1 goat which had become infested with *Oesophagostomum* spp. on pasture were examined and all were identified as *O. columbianum*. The position of the cervical papillae, approximately at the level of the middle of the

esophageal region in fourth stage *O. columbianum* and at the level of the base of the esophageal region in fourth stage *O. venulosum,* can be used to distinguish the two species.

The minor adhesions noted at postmortem may have been the consequence of a slight enteritis due to the activities of the worms.

None of the animals were immune to an initial infection with *O. venulosum.* However, the intestine of some animals appeared to be more easily irritated and the worms soon scoured out. This is perhaps exemplified best by the history of lamb A1 (table 3).

In general, animals more than a year old when first infected developed fewer adult worms or tended to eliminate them more rapidly than younger animals. This is exemplified by sheep 940 (table 3) and 993 (table 2). In older animals stronger peristaltic action, the secretion of greater quantities of mucus, and increase in the bulk of intestinal contents probably allowed fewer worms to become established in the intestine, or caused them to pass out sooner.

As indicated by the egg output, fewer mature worms became established in reinfections, after the termination of initial infections, than in initial infections. This is exemplified in the histories of lambs 907, 959, A1, A12 (table 3), yearling 940 (table 3), and lamb L-989 (table 4). Usually the greater the number of larvae administered in the initial infection and the more extended the period of administration of the larvae, the greater was the resistance to reinfection after the termination of the initial infection. The histories of lambs A1 (table 3) and A18 and A16 (table 6) may be taken as examples. Lamb A1 had been fed 4,200 larvae by single inoculation in the initial infection. In reinfection, after the termination of the initial infection, this lamb showed evidence of infection in a soft stool, but the worms were eliminated before they could pass any eggs. Lambs A18 and A16 had been fed 28,000 larvae in 94 days, and 4,400 larvae in 23 days, respectively, in the initial infections. Four months after lamb A18 had last passed eggs in its feces, and 2 months after lamb A16 had last passed eggs, each was fed 500 larvae. At the same time a previously uninfected control lamb, A61 (table 6), was also fed 500 larvae from the same batch. At necropsy, a month later, 457 worms were recovered from lamb A61, 31 from lamb A16, and only 1 from A18.

As indicated by the worms recovered from lambs 975, A15, A61, and A37 (table 1) in light to moderate initial infections almost all the worms became adults and matured within one month after infection. In kid G-777 (table 3), after reinfection with a somewhat heavy dose of larvae (4,500), following the termination of a moderate initial infection, 90 per cent of the worms recovered at necropsy, 39 days after reinfection, were still in the fourth stage. This may have indicated resistance of the host to development of the worms as a result of the initial infection.

SUMMARY AND CONCLUSIONS

1.—As indicated by the egg output, more mature adult *Oesophagostomum venulosum* became established in the intestine when 350 to 1,500 larvae were administered in a single inoculation than in greater or lesser inoculations, or when larvae were administered over a period of time.

2.—The longest period during which eggs were passed in the feces of any of the experimental animals was approximately 400 days.

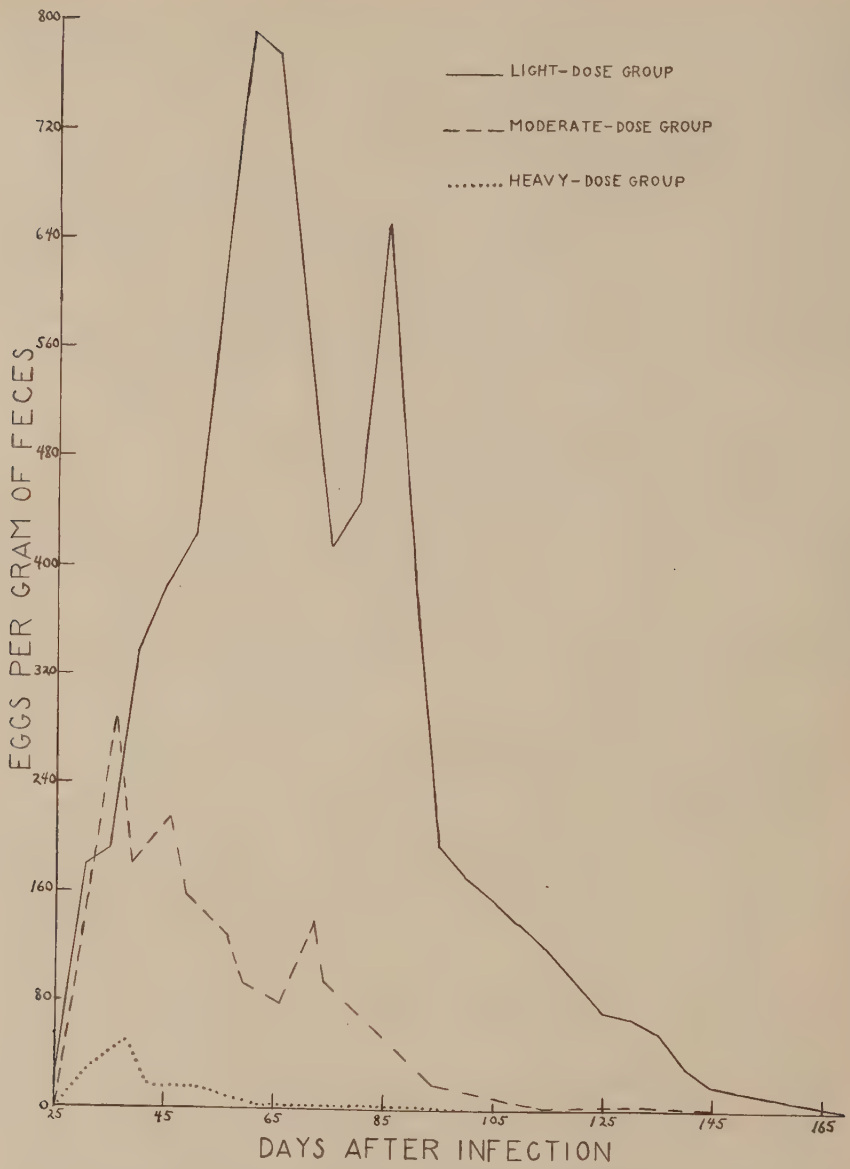

FIG. 1. Egg counts following light doses of larvae (average of 1,817 larvae per animal, single inoculation, 4 lambs and 3 kids), moderate doses (average total 4,367 larvae per lamb, in 6 inoculations during 23 days, 3 lambs), and heavy doses (total 28,000 larvae per lamb, in 31 inoculations during 94 days, 3 lambs).

3.—The symptoms noted included diarrhea, unduly foul feces, and the passing with the feces of excessive amounts of mucus occasionally specked or streaked with blood. The freshness of the blood indicated its source from the cecum or remainder of the large intestine, the locations of mature *O. venulosum*.

4.—The symptoms were caused by the adult worms. The administration of moderate numbers of larvae, 500 or more, was usually sufficient to cause symptoms.

5.—When more than 4,000 larvae were administered, the effects on the host were sufficient to interfere with weight gain to a slight extent.

6.—Few or no lesions were noted at postmortem. These included petechiae, nodules 1 to 2 mm. in diameter, and minor adhesions. The maximum number of lesions was observed in the animal necropsied 13 days after infection. It had approximately 200 yellowish, necrotic, ulcerative lesions 1 to 2 mm. in diameter in the small intestine. The maximum number of calcareous lesions was 10. If these were due to *O. venulosum* larvae, they were the rare exception to the usual course of events. No larvae were observed in any of the lesions. The larvae did not remain in the wall of the intestine long enough to induce the formation of serious local lesions.

7.—The symptoms and postmortem findings were not nearly as serious as those in animals with comparable infections of *O. columbianum.*

8.—Individual natural resistance was at least as significant as the number of larvae administered in determining the number of mature worms which became established in the intestine. However, none of the animals were completely immune to an initial infection. Those most susceptible to the effects of the worms soon scoured them out.

9.—Older animals appeared to be slightly more resistant to infection than young animals.

10.—As indicated by the egg output, fewer mature worms became established in the intestine in reinfections, after the termination of initial infections, than in initial infections. Partial or complete resistance to reinfection developed as a result of the initial infection. In reinfection, in one case, the worms remained immature for a longer period than in the animals necropsied after an initial infection. This may be another indication of resistance of the host resulting from an initial infection.

11.—The greater the number of larvae administered in the initial infection, and the more extended the period of administration of the larvae, the greater was the resistance to reinfection after the termination of the initial infection. One lamb retained its resistance to reinfection for at least 4 months.

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OBSERVATIONS ON *PEREZIA PYRAUSTAE* PAILLOT, A MICROSPORIDIAN PARASITE OF THE EUROPEAN CORN BORER[1]

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A considerable number of diseased specimens of the European corn borer, *Pyrausta nubilalis* (Hübner), were received during 1950 by the Laboratory of Insect Pathology (Division of Biological Control, University of California) from K. D. Arbuthnot of the U. S. Department of Agriculture, European Corn Borer Research Laboratory, Ankeny Field Station, Des Moines, Iowa, where general research on the European corn borer is being conducted under the supervision of W. G. Bradley. Examination and study of these specimens by E. A. Steinhaus (1951*a*) of our laboratory revealed the presence of several different pathogens (fungi, bacteria, and protozoa), among them a species of MICROSPORIDIA. The data relative to the fungi and bacteria are the subject of another report (Steinhaus, 1951*b*).

Since MICROSPORIDIA are obligate parasites, and since the European corn borer is not present in California, it was necessary, in order to maintain the protozoan, to find a suitable host among the local insects readily available and adaptable to insectary rearing. The buckeye caterpillar, *Junonia coenia* Hübner, proved to be such a host. Preliminary cross-infection tests were accomplished by feeding the caterpillars plantain dipped in a spore suspension of the microsporidian from one corn borer larva. This specimen, collected in Black Hawk County, Iowa was one of 46 received by the laboratory on August 5, 1950. After 12 days, two of the four test *Junonia* larvae were found to contain microsporidian spores and vegetative stages, while the three control larvae were not infected.

A second cross-infection test was made using a greater number of *Junonia* larvae. The spore material used to contaminate the insect food was prepared from four European corn borer specimens from a shipment of 32 received on August 8, 1950. Two of the specimens were collected in Henry County, Iowa, one in Boone County, Iowa, and one in Bremer County, Iowa. Three specimens contained only microsporidia while one contained both microsporidia and a *Beauveria* fungus. Slides were made on the sixth, eighth, eleventh, and thirteenth days after the *Junonia* larvae were fed the contaminated food. The slides were fixed with methyl alcohol and stained with Giemsa. Vegetative stages were evident on the eighth day, mixed vegetative stages and spores on the eleventh day, and mostly spores were present on the thirteenth day.

Observations on the life history of the microsporidian revealed that each sporont forms two spores. Since the infected cells of the *Junonia* larvae are not hypertrophied, this microsporidian, according to the present systematic concepts, may be placed in the genus *Perezia* Léger and Duboscq, 1909. This brings forth a dubious aspect of classification of the MICROSPORIDIA in which two genera (*i.e., Glugea* Thélohan and *Perezia* Léger and Duboscq are distinguished not on the basis of their own characteristics but upon pathological changes occurring within the host

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tissues. Of interest would be cross-infection tests involving species of *Glugea* with known hosts of species of *Perezia* and vice versa to determine if the concept of pathological changes in the host is a valid basis for separating the two genera. Were such host pathological changes proven to be of no taxonomic significance, the law of priority would force the mergence of the two genera into one under the name *Glugea*.

Two species of Microsporidia have been reported to infect the European corn borer. Paillot (1928) found a microsporidian, which he named *Perezia pyraustae*, to infect larvae of the European corn borer in France, while Kotlán (1928) described as *Nosema pyraustae* a microsporidian isolated from the same host. Recognizing the lack of adequate information to determine possible synonymy with certainty, Steinhaus (1949) has suggested that *Nosema pyraustae* Kotlán may possibly be the same species as *Perezia pyraustae* Paillot.

A study of the life cycle of the microsporidian herein described reveals only minor differences between this organism and *Perezia pyraustae* as described by Paillot. The early schizont stage of this microsporidian is represented by a body about 3 microns in diameter with a single compact nucleus in the center of a uniformly staining cytoplasm (Fig. 1). Other early stages are evident with the nuclear material more diffused and surrounded by a clear zone (Figs. 2–3). Nuclear division appears to take place during this diffused state with the more common binucleate condition resulting (Fig. 4). A second nuclear division produces a tetranucleate body (Figs. 5–6) which in turn divided by binary fission into two daughter binucleate cells, each about 4 microns in diameter (Figs. 7–8). Rarely found are multinucleate chains about 10 to 12 microns long with from 4 to 8 nuclei grouped in pairs (Figs. 9–10). These chains appear to divide by binary fission into the binucleate forms. In a few instances 4-, 6-, and 8-nucleate cells were observed (Figs. 11–13). Although lines of cleavage in the cytoplasm were not noted, the peripheral position of the nuclei in most cases would suggest the possibility of multiple division taking place with uninucleate and binucleate forms resulting. In some multinucleate cells, the nuclei appear to have clear centers (Fig. 6).

The schizont stages as described above compare favorably with those of *Perezia pyraustae* with a few exceptions. The multinuclear elements of *P. pyraustae* according to Paillot never contain more than four nuclei while as many as eight nuclei may be present in the schizont chains of this microsporidian. The peripheral position of the nuclei in some cells and the clear centers of the nuclei were not mentioned by Paillot.

The early sporont stage is a body about 9 microns long, binucleate and with a lightly-staining vacuolated cytoplasm (Fig. 14). As in the schizont stages, when the nuclei are compact and stain deeply, there is often a clear spot in the center of each nucleus. After nuclear division takes place resulting in a tetranucleate body (Fig. 15), the sporont divides by binary fission into two binucleate sporoblasts, each about 5 to 7 microns long (Figs. 16–17). The sporoblasts usually separate before spore formation, but occasionally double spores are formed (Figs. 25–26) indicating that complete separation of the two sporoblasts does not always take place. Although the typical sporoblast is binucleate, one was observed with four compact nuclei, indicating a possible rearrangement of the nuclear material prior to spore formation (Fig. 18).

The spores vary in size from 3.5 to 6.0 microns in length and 1.8 to 3.0 microns


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in width, averaging about 4.5 microns long by 2.0 microns wide. While curved spores are rather common (Fig. 21), double spores about 8 microns long by 3 microns wide (Fig. 26) and L-shaped and T-shaped spores are formed infrequently (Figs. 22–23). In occasional well-stained spores, two nuclei appear to be centrally located in the sporoplasm indicating a binucleate state (Fig. 20). Some spores have two compact nuclei at the anterior end while others have one compact nucleus in this location (Figs. 27–28). Apparently the two nuclei migrate to the anterior end of the sporoplasm where they fuse. This phenomenon has been observed also in the life cycle of an unidentified species of *Nosema* from the sod webworm, *Crambus bonifatellus* (Hulst), and fits in well with the beginning of the life cycle where the first stage is uninucleate.

Extrusion of the polar filament from the more slender end of the spore was accomplished using Gram's iodine solution as an irritant (Fig. 24). No extrusions were observed using pressure. Length of the polar filament varied from 30 to 65 microns.

The sporont stages and normal spores of this microsporidian are similar to those of *P. pyraustae* and both form oversize and double spores. Paillot did not describe the presence of curved and T-shaped spores nor did he give the length of the polar filament. Therefore, comparison cannot be made in this regard. When spore suspensions from smears of larvae of the corn borer are examined, the curved spores are found to occur, while no T-shaped spores are present. The rare T-shaped spore might possibly be formed only in such an alternate host as the buckeye caterpillar where conditions differ from those in the natural host. No description was made by Paillot of the anterior migration and fusion of the spore nuclei resulting in a uninucleate spore.

The principal site of infection in both cases is the malpighian tubules. Apparently the adipose tissues of the European corn borer are not parasitized by *P. pyraustae* while the microsporidian with which we are concerned does invade the fat tissues of the buckeye caterpillar.

A comparison of the microsporidian with the descriptions of the other reported species of *Perezia* reveals differences sufficient to distinguish it from any of them. While the length of the polar filaments of this microsporidian varies from 30 to 65 microns, those of *Perezia mesnili* Paillot (1918*a*) are estimated to be about 18 to 20 microns long. The polar filaments of *Perezia legeri* Paillot (1918*b*), while about 30 to 40 microns in length, are extruded from the rounded ends of the spores. The schizonts of *Perezia pieris* Paillot (1924) apparently have no multicellular chains with more than two binucleate cells and the spores are first uninucleate, then become binucleate. The microsporidian from the European corn borer has rare multicellular chains with from 4 to 8 nuclei and the spores are usually binucleate, becoming uninucleate before germination. According to Weiser (1946), the extruded polar filaments of *Perezia trichopterae* Weiser measure 200 to 300 microns in length and the spores often are attached in a side-by-side position.

Since there are only minor differences between this organism and *Perezia py-*

PLATE 1. Stages in the life cycle of *Perezia pyraustae* Paillot. Figs. 1–13, schizonts; Figs. 14–18, sporonts; Fig. 19, young spore; Fig. 20, mature spore; Fig. 21, curved spore; Figs. 22–23, abnormal spores; Fig. 24, spore with fully extruded polar filament; Figs. 25–26, double spores; Figs. 27–28, binucleate and uninucleate spores.

raustae Paillot as described by Paillot from diseased specimens of the European corn borer in France, the two microsporidia may be considered to be the same or varieties of the same species. According to Metcalf, Flint, and Metcalf (1951), *Perezia pyraustae* has been used in attempts to control the European corn borer in the United States. Sweetman (1936) reported *P. pyraustae* to have been found in the United States as well as in Europe although he gave no authoritative source for the statement. Since, on the basis of recent correspondence with Dr. Robert Metcalf and Dr. Sweetman, it may be assumed that the statements resulted from misinformation, the present report constitutes the first indication that *Perezia pyraustae* Paillot is a parasite of the European corn borer in the United States.

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AN ADDITIONAL NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *NEOECHINORHYNCHUS*

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During a survey of the parasites of the river carpsucker (*Carpiodes carpio*) of Lake Texoma, made at the University of Oklahoma Biological Station, the junior author of this paper found numerous specimens of the acanthocephalan genus *Neoechinorhynchus*. These individuals differ in several points from all the previously described species of that genus. The worms were submitted to the senior author for critical study. Extended observations verified the tentative conclusion that this material represents a previously unrecognized species which is here described as *Neoechinorhynchus prolixus* n. sp.

This is the twentieth species of the family NEOECHINORHYNCHIDAE and the twelfth species of the genus *Neoechinorhynchus* known from North American fishes. While the members of this genus are not sharply restricted in their host relations on this continent, they are preeminently associated with the members of the CATOSTOMIDAE or suckers. Seven of the twelve species of *Neoechinorhynchus* now known from North America utilize catostomids as definitive hosts. As has been pointed out in an earlier paper (Van Cleave 1949) it is obvious that diversification of the catostomids on this continent has been accompanied by an extremely active speciation of their acanthocephalan parasites. On no other continent has there been such a conspicuous eruptive evolution of the NEOECHINORHYNCHIDAE. The available records tend to show that some species are practically host specific and of extremely limited geographical distribution. At least in some instances this impression is due to scarcity of data and complete absence of either positive or negative records for large areas of the country. Some of the species will doubtless be found to have broader host tolerance and wider geographical distribution when the parasite fauna of other hosts become better known, since among the ACANTHOCEPHALA similarities in food habits and in habitat of the hosts are recognized as favorable for extension of the host list. However, it is likewise probable that absence of these favorable conditions may form the basis for establishment and maintenance of distinct host specificity for some species.

Of 201 individuals of *Carpiodes carpio* (Raf.) examined from Lake Texoma, 155 were infected by *N. prolixus*. These yielded a total of 1123 specimens, with the heaviest infection consisting of 80 individuals. No other species of ACANTHOCEPHALA was found in this host of Lake Texoma, nor was *N. prolixus* found in any other host. However, an exhaustive survey of the parasites of the fishes has not yet been completed. Extended studies of *Ictiobus bubalus* (Raf.) (the smallmouth

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[1] Part of the material included in this paper will be used by the junior author in a thesis for the Master of Science degree, University of Oklahoma, under the direction of Dr. J. Teague Self.

buffalo) and of *Lepibema chrysops* (Raf.) (the white bass) in this same lake were all negative for *N. prolixus*.

Neoechinorhynchus prolixus n. sp.
Figs. 1–6

Description: With the characteristics of the genus *Neoechinorhynchus* as diagnosed by Van Cleave (1919). Body long and narrow, length from 16 to 22 times the maximum diameter. Preserved individuals without conspicuous specialization of any body region (Fig. 1), practically cylindrical in cross section, tapering very gradually posteriorly from the level of the ventral subcuticular giant nucleus and more rapidly anteriorly to meet the narrowed neck. Proboscis (Figs. 3 and 4) small, usually slightly broader than long. Binucleate lemniscus (Fig. 2) much longer and broader than the uninucleate, in males its posterior end characteristically terminating some distance anterior to the anterior testis. Dorsal body wall, especially in females (Fig. 5), considerably thicker than the ventral wall.

Females: 7 to 16 mm. long with maximum diameter of from 0.42 to 0.99 mm. Proboscis 0.098 to 0.112 mm. long by 0.098 to 0.140 mm. broad. Hooks of terminal series 0.042 to 0.056 mm. long; those of middle and basal series of practically identical length (usually 0.028 mm.). Binucleate lemniscus often approximately 1/4 to 1/3 the body length; uninucleate lemniscus usually less than 1/2 the length of the binucleate and always very conspicuously narrow.

Shelled embryos in preserved females 0.026 to 0.032 in length by 0.010 to 0.015 mm. in width. The junior author of this paper took measurements of living eggs in water and found them to range from 0.033 to 0.043 mm. in length by 0.015 to 0.025 mm. in width. This evidence of shrinkage of eggs of *Neoechinorhynchus* upon preservation is in keeping with the findings of Lynch (1936). The copulatory cap shown in Fig. 7 is abnormal in that shelled embryos are embedded in the cement, giving proof that the eggs of this female had been fertilized prior to the last copulation.

Males: 5.5 to 11.9 mm. long, with maximum diameter of 0.35 to 0.57 mm. Proboscis 0.084 to 0.112 mm. long (commonly 0.098 mm.) by 0.098 to 0.126 mm. broad. Proboscis hooks not appreciably different from those of female. Both lemnisci usually terminating anterior to the first testis. Male genital complex frequently about 1/2 the length of the body cavity. Cement gland (Fig. 1) from about 1 to over 2 mm. long, with 8 nuclei. Copulatory bursa about twice as long as wide when extruded.

Type Material: Holotype, male, slide VC 4509.8 and allotype female, slide VC 4509.1 in the collection of Harley J. Van Cleave, Urbana, Illinois. Paratypes of both sexes in United States National Museum, the Museum of the University of Oklahoma, the slide collection of the Oklahoma Biological Station and the private collections of the two authors of this paper.

Definitive Host: In the intestine of *Carpiodes carpio* (Raf.), the carpsucker of Lake Texoma, Oklahoma. Developmental stages unknown. Living worms, in the intestine of the host, are opaque and yellowish-white in color. In water they become relatively translucent, milky white.

Comparisons: *N. prolixus* belongs to that group of species of the genus *Neoechinorhynchus* in which there is extreme size diversification of the two lemnisci (*N. cristatus, N. venustus, N. australis* and *N. distractus*). In features of the proboscis and its armature, it has many points in common with *N. cristatus*. However, the mature worms of *N. prolixus* are much longer (males to 12 mm., females to 16 mm.) than measurements recorded for *N. cristatus* (males to 3.55 mm., females to 10 mm.). Since the females of *N. cristatus* are fully mature, it is evident that the size difference cannot be attributable to differences in growth conditions

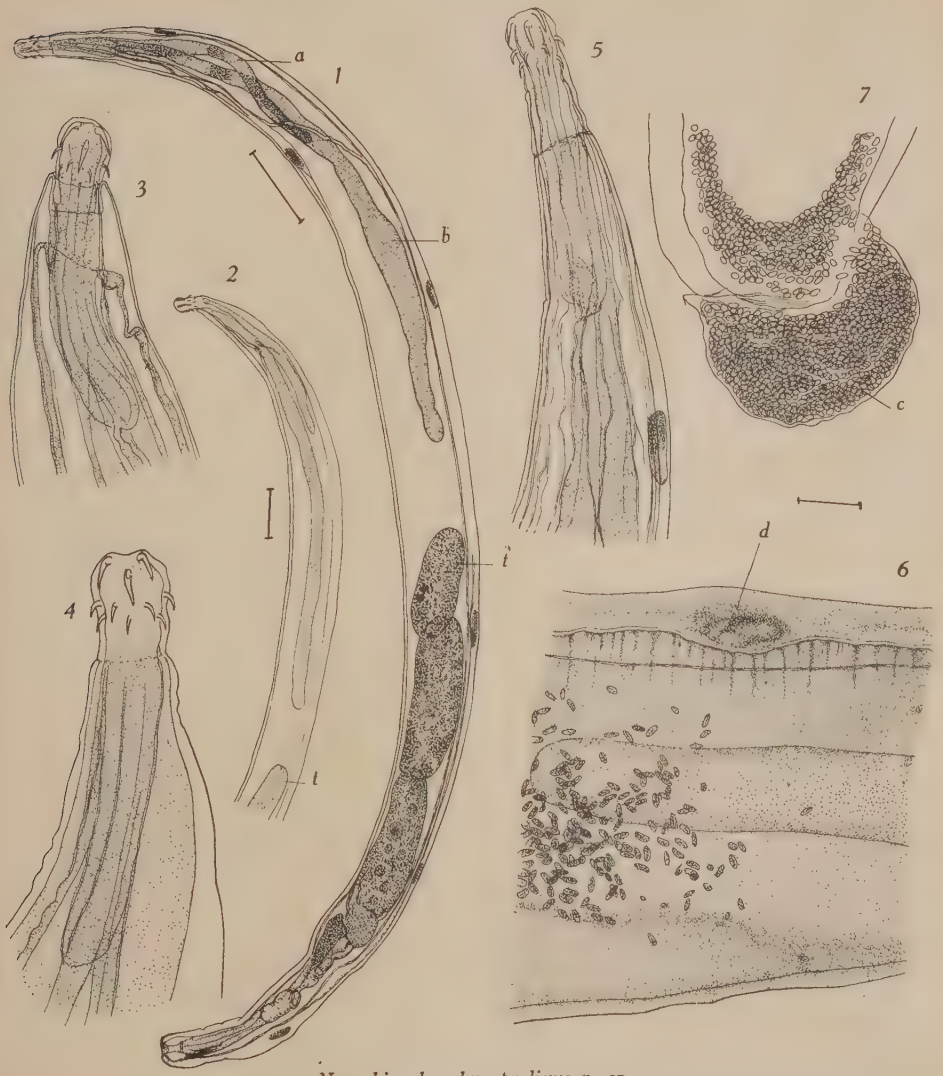
EXPLANATION OF PLATE

All drawings, from stained permanent mounts by use of a camera lucida, were prepared by Katharine Hill Paul, Scientific Artist in the Department of Zoology, University of Illinois. The scale of magnification beside Fig. 1 has the value of 0.5 mm., and applies to this figure only; that near Fig. 2 is 0.5 mm., and applies to this figure only. The scale between Figs. 6 and 7 is 0.1 mm., and applies to Figs. 3 to 7.

Symbols

a—uninucleate lemniscus b—binucleate lemniscus
c—copulatory cap d—a dorsal subcuticular nucleus
t—testis

PLATE I



Neoechinorhynchus prolixus n. sp.

FIG. 1. Entire paratype male, side view, showing all organs.

FIG. 2. Anterior end of holotype male, showing relations of lemnisci.

FIGS. 3 to 5. The proboscis, showing arrangement and size relations of the hooks; 3, paratype male; 4, allotype female; 5, paratype male.

FIG. 6. A characteristic region of the trunk of a paratype female, showing differences in thickness of dorsal and ventral walls.

FIG. 7. Posterior extremity of paratype female, showing copulatory cap with embryos embedded in it.

in their respective recorded hosts. Furthermore, the eggs of preserved females of *N. prolixus* (0.026 to 0.032 by 0.010 to 0.015 mm.) are smaller than those of preserved *N. cristatus* (0.038 by 0.016 mm.) and this fact adds significance to the observation that smaller body size in *N. cristatus* could not be attributable to immaturity. The most pronounced difference between these two species is in the relative proportions of their bodies. In *N. cristatus,* the length of the body is approximately 10 times the maximum diameter while in *N. prolixus* body length is more often about 20 times the diameter. In males, this disparity in body proportions is reflected in the extent of the reproductive organs and their spatial relations to the lemnisci. In *N. cristatus,* the cement gland averages 0.623 mm. in length (Lynch, 1936) while in *N. prolixus* it may exceed 2 mm. The long, binucleate lemniscus in *N. cristatus* extends backward from the neck to near the anterior margin of the posterior testis while in *N. prolixus* (Fig. 2) there is usually considerable distance between the posterior end of the long lemniscus and the front margin of the anterior testis.

The dorsal body wall of females of *N. prolixus* has a tendency toward thickening (Fig. 5) as described for *N. cristatus* (Lynch, 1936), but in the specimens under observation the thickening is not so extreme in *N. prolixus.*

N. prolixus differs from *N. venustus* in that the proboscis of the new species is markedly smaller in both length and breadth than that of *N. venustus* and the proboscis hooks of the new species are distinctly smaller than those of *N. venustus.* Furthermore, there is but slight distinction in length between the basal and middle series of hooks in *N. prolixus* (Figs. 3, 4) while in *N. venustus* hooks of the middle series are conspicuously longer than those of the basal series. The embryos in preserved specimens of *N. venustus* (0.043 to 0.062 mm. by 0.022 to 0.026 mm.) average approximately twice the dimensions of eggs from similar specimens of *N. prolixus* (0.026 to 0.032 by 0.010 to 0.015 mm.).

N. prolixus differs from both *N. australis* and *N. distractus* in the sizes of the proboscis hooks. This is particularly true for the length of the middle series of hooks since in *N. australis* and *N. distractus* hooks of the middle series are distinctly longer than those of the basal series. The relations of the male organs to length of the body cavity and to the long lemniscus in *N. prolixus* are somewhat similar to conditions found in *N. distractus* but are unlike conditions found in *N. australis.*

The proboscis of *N. prolixus* has a general appearance similar to that of *N. rutili.* However, the greater size and proportions of the body, extent of the male organs, and disparity in size of the lemnisci in *N. prolixus* set that species off sharply from *N. rutili.*

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ARIOLIMAX COLUMBIANUS, AN INTERMEDIATE HOST FOR *BRACHYLAEMUS VIRGINIANA* (DICKERSON) (TREMATODA: DIGENEA) IN CALIFORNIA

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On March 3, 1951 a young male opossum was captured in Tilden Park, Contra Costa Co., California. The animal had a droopy, sickly appearance and was killed and examined for parasites. Several hundred small flukes, later identified as *Brachylaemus virginiana* (Dickerson), were found in the small intestine. Several months previously one of us had found metacercariae in the kidney of *Ariolimax columbianus*. This slug is commonly found in the Pacific Coast region and is restricted to this area. Comparison of the metacercariae with the flukes obtained from the opossum, as well as examination of pertinent literature (Dickerson 1930, Krull 1935), indicated clearly that the larvae from the slugs were metacercariae of *Brachylaemus virginiana*.

Of 97 *Ariolimax columbianus* collected on the Mills College Campus, Alameda Co., California, 94 harbored this metacercaria in the kidneys. There were approximately two dozen larvae per kidney, while specimens with as few as one or two and others with as many as 50 or more were observed. In addition, 6 out of 8 *Ariolimax columbianus* from Berkeley, Alameda Co., California were infected. Seven *A. californicus brachycephalus* from San Mateo Co., California were found to be negative. Although the digestive, hermaphroditic and albumen glands were examined in several specimens of *A. columbianus*, no sporocysts or cercariae were found.

Krull (1936) listed 1 slug and 5 snail intermediate hosts for *Brachylaemus virginiana*. We therefore examined a number of common gastropods, in order to determine possible additional hosts for the larval stages of this fluke. Of 20 *Milax gagates*, 10 *Limax marginatus*, 1 *Limax flavus*, 20 *Deroccras reticulatum* and 6 *Helminthoglypta arrosa holderiana* not a single one was infected. All these gastropods were collected on the Mills College Campus between February and May 1951 in the same habitat with *Ariolimax*. Three *Helminthoglypta nickliniana* from Tilden Park, Contra Costa Co., California, were also negative. Since the slug *Deroceras laeve* is a host for the metacercaria (Krull, 1936) one would expect that *D. reticulatum* might also be suitable. This was not so, however, and it was somewhat surprising to find the metacercariae only in *Ariolimax*. It may be that the food habits of the above mentioned gastropods are in part responsible for this situation. *Ariolimax columbianus* has been observed to congregate around and feed on feces in nature. None of the other species listed has been observed to do so.

In relation to the opossum in California, we should like to stress that this animal was introduced into the state, the earliest record being sometime after 1880 (Grinnell, Dixon and Linsdale, 1937); subsequently it was introduced repeatedly around

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the turn of the 20th century. With the introduction of the opossum into a new area, it was essential for the survival of its parasites or, at least, *B. virginiana* that a suitable intermediate host be present. *Ariolimax,* strictly a Pacific Coast species, must have proved immediately capable of serving as a new intermediate host. Without its presence, the survival of *B. virginiana* on the West Coast would have been difficult if not impossible, judging from the data presented above.

Similar problems are encountered by any parasite which requires an intermediate host for the completion of its life cycle, and the successful introduction and establishment of such parasites in new areas will depend on the presence of a suitably preadapted intermediate host in the new habitat of the definitive host.

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MESOZOAN PARASITES OF *OCTOPUS VULGARIS*, LAM. FROM FLORIDA[1]

BAYARD H. MCCONNAUGHEY[2] AND HENRY KRITZLER[3]

Dicyemid mesozoans have been found to infect littoral cephalopods of Europe, western United States, and Japan. They have been found wherever the littoral cephalopods have been examined for them except in the Pacific Oceanic Islands. It is surprising that there have been no reports of this group from the eastern seaboard of the Americas.

In January 1948, while employed by Marine Studios Inc., near St. Augustine, Florida, one of us (H. K.) discovered that specimens of *Octopus vulgaris* on display in the aquarium were infected with these parasites. Several sets of slides were prepared and sent to the senior author. The following summer both of us collected additional material.

We wish to acknowledge our indebtedness to the management of Marine Studios Inc. for the many courtesies extended and for the facilities put at our disposal, and to Mrs. Evelyn McConnaughey for technical assistance.

MATERIALS AND METHODS

The octopuses were taken by collectors from Marine Studios Inc. in approximately twelve fathoms of water about fourteen to fifteen miles offshore from St. Augustine. Five of them were killed immediately upon arrival at the aquarium. Smear preparations were made from their kidneys, fixed in Bouin's fluid and stained with Ehrlich's acid hematoxylin. Other preparations were made from octopuses on display in the aquarium as soon as possible after their death. Degeneration of the kidney tissue and of the mesozoans begins soon after the death of the host, making the study of such material more difficult. However, specimens satisfactory for determination were obtained from eight such octopuses, making a total of thirteen.

About sixty small squids taken offshore in nets were also examined but were found not to contain MESOZOA.

DESCRIPTION OF SPECIES

Two species of dicyemids, both members of the genus *Dicyema,* were found. One of these appears to be *D. typus* van Beneden, as restricted by Nouvel (1947). The other is a new species for which the name *Dicyema aegira* is proposed. In nine of the octopuses examined only *D. aegira* was found, three contained both species, and one appeared to be infected only with *D. typus.*

Dicyema aegira n. sp.

(Figs. 1–3)

Diagnosis: Small to medium sized dicyemids, adults mostly 0.4 to 1.5 mm. long; body of approximately same width throughout except for a moderate cephalic swelling broadest at the

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level of the parapolar and first diapolar cells; somatic cells 22 in number, seldom varying from this, though a few individuals with other numbers (21 to 25) have been found; calotte orthotropal, slightly longer than broad, not exceeding the anterior end of the trunk in breadth, only slightly tapering to the broadly rounded anterior end; propolar cells smaller than the metapolar cells; axial cell ending bluntly at the base of the calotte, not extending forward to the propolars; trunk cells in opposed pairs, not conspicuously charged with granules; no verruciform cells except the paired uropolars which may exhibit this condition in slight degree; vermiform larvae 40 to 50 microns long at the time of their liberation, containing two axoblasts, one anterior and one posterior to their axial nucleus.

Rhombogens most commonly with only one or two infusorigens, usually located in the anterior half or third of the body.

Infusorigens often small, producing only a few egg cells at a time, though exceptions have been noted.

Infusoriform 30 to 35 microns long, with relatively small refringent bodies; urn large, each of the four cells within the urn containing two free nuclei and one germ cell.

Chromosome number: diploid number estimated to be 16 or 18.

Host: Octopus vulgaris Lamarck.

Loc.: Offshore about 14 miles from St. Augustine, Florida, in approximately 12 fathoms of water.

Type specimens: Syntypes on slide F2-36. Paratypes on other slides of the F2 series.

This species shows a pronounced tendency to produce accessory nuclei in the posterior diapolar and uropolar cells at or slightly before the onset of the rhombogen phase. The axial nucleus also sometimes divides amitotically so that the axial cell may contain one or more free nuclei in addition to the original axial nucleus and the paranuclei.

The infusorigens and their products are usually located well forward in the anterior portion of the rhombogen, causing the swelling behind the calotte to be more pronounced in this phase (Fig. 2). The growth of the infusoriform appears to be rapid, judging from the facts that embryonic stages do not occur in any numbers and that only two or three infusoriforms are usually present at any time in a given rhombogen.

D. aegira resembles *D. acciaccatum* McConnaughey, in cell number and in the fact that the axial cell does not penetrate forward in the calotte to the base of the propolar cells. *D. acciaccatum* is a smaller species, seldom exceeding 0.8 mm. in length. It shows less tendency to have the contents of the axial cell crowded into the anterior part of the body in the rhombogen phase. The uropolar cells are less granular, not different in character from the other trunk cells. It was taken in a shallow intertidal area at Balboa Bay in southern California from an undetermined

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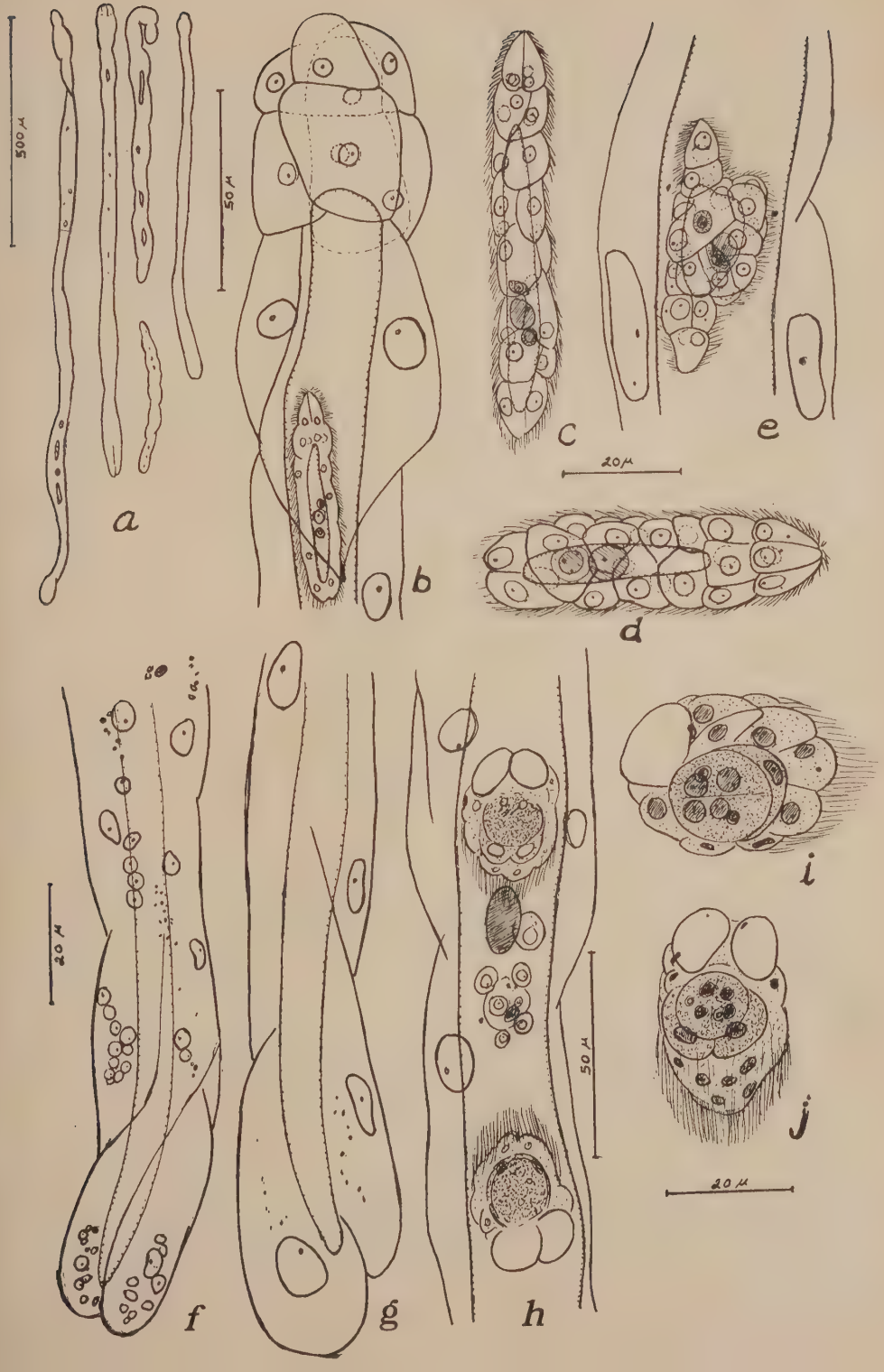
FIG. 1. *Dicyema aegira.*

a. Group of individuals drawn to the same scale to show general form and size.
b. Anterior end of a large nematogen to show the form of the calotte and its relation to the axial cell.
c. Normal vermiform larva, fully developed, showing the characteristic number and arrangement of cells.
d. Younger vermiform larva before the division of the first axoblast.
e. Abnormal vermiform larva with divided calotte, the two half-calottes pointing in opposite directions.
f. Posterior portion of the trunk of a rhombogen showing fragmentation of the nuclei of the somatic cells.
g. Posterior end of the trunk of a young nematogen for comparison with the condition in f.
h. Portion of a rhombogen showing small infusorigen and two fully developed infusoriform larvae.
i. Slightly oblique parasagittal optical section of an infusoriform larva.
j. Frontal optical section of an infusoriform larva.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octopus (possibly *O. bimaculoides* Pickford and McConnaughey). *Octopus vulgaris* is not known to occur in that area.

Since the description of *D. acciaccatum* is based on dicyemids from only one octopus it is impossible to state with certainty that these small differences are constant. However, since the two groups of dicyemids were taken in different hosts from widely separated geographical areas and in different ecological situations, and since in the limited material available there are some significant differences, it seems best to regard them as separate species.

The other species of *Dicyema* known to have the axial cell end at the base of the calotte instead of continuing forward to the base of the propolar cells all differ from *D. aegira* in the number of somatic cells characteristically present as well as in other characters.

Teratology: One curious vermiform larva, fully developed and with the characteristic number of somatic cells, was found, in which the calotte had become divided longitudinally during early development. This resulted in two half calottes, each consisting of two propolar and two metapolar cells. The two half calottes were

FIG. 2. *Dicyema aegira.* Group of rhombogens showing typical disposition of contents of the axial cell in the anterior part of the body with resulting swelling of this region.

separated by several trunk cells and were pointing in opposite directions, giving the appearance of a two-headed embryo (Fig. 1, e).

One giant size infusoriform larva was found, approximately double the normal dimensions. The contents of the urn appeared to have stretched the ventral pore wide open, and each of the four cells within the urn exhibited a curious aggregation of the stainable cytoplasm in a crescent on one side, leaving the two free nuclei and the germ cell in what appeared to be a large vacuole (Fig. 3).

Dicyema typus van Beneden

(Fig. 4)

As restricted by Nouvel, *D. typus* is a short, stocky species with only 18 or 19 somatic cells in most individuals, none of them verruciform, and with the axial cell penetrating the calotte to the base of the propolars. The infusoriform has large refringent bodies. It has been reported from *Octopus vulgaris* along the shores of the North Sea, the Atlantic coast of France, and the Mediterranean.

SUMMARY

Two species of *Dicyema* were taken in a series of thirteen octopuses from the coast of Florida in the vicinity of St. Augustine. One of these appears to be *D. typus* van Beneden, as restricted by Nouvel. The second is described as a new species for which the name *Dicyema aegira* is proposed.

A series of about sixty small squids taken in nets from this area failed to show any evidence of infection with dicyemids.

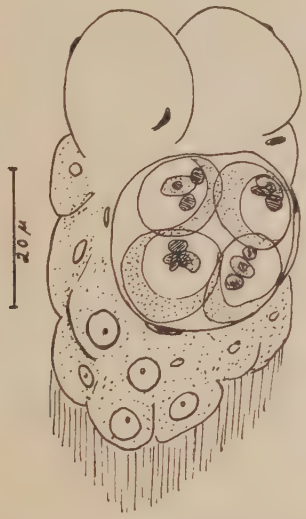

FIG. 3. *Dicyema aegira*. Abnormal giant infusoriform larva approximately double the usual size. The ventral pore is stretched open by the contents of the urn and the cytoplasm of the cells within the urn is gathered in a crescent leaving the nuclei and germ cell of each in a large vacuole.

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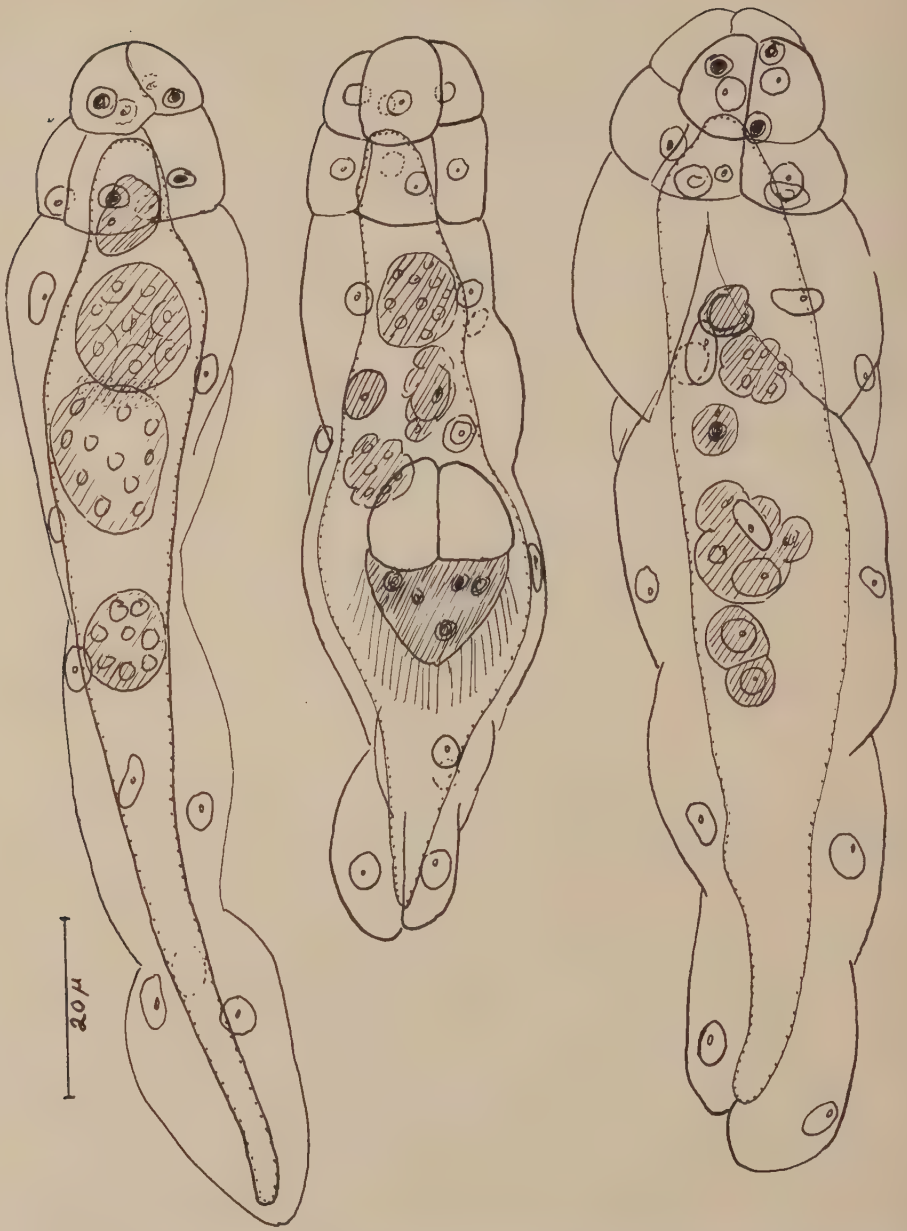

FIG. 4. *Dicyema typus*. Three rhombogen individuals showing typical appearance and cell number. The nuclei of some of the cells of the calotte in many individuals appeared pycnotic. The rhombogens did not have accessory nuclei in the somatic cells of the trunk.

LABORATORY EVALUATION OF ORGANIC COMPOUNDS AS MOLLUSCACIDES AND OVOCIDES, II

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A preceding paper (Batte, Swanson and Murphy—1951) reported results of screening 33 compounds for their molluscacidal activity against *Pseudosuccinea columella* Say and *Fossaria cubensis* Pfr., the intermediate hosts of liver flukes in Florida. Copper sulfate and dinitro-o-cyclohexylphenol were the only compounds which gave 100 per cent mortality of the test snails following 24 hours exposure to concentrations of 1 : 1,000,000 and stronger.

The previous paper deals with the literature on the subject, methods used, and gives some of the results. This paper presents results with other compounds.

MATERIALS AND METHODS

Compounds which exhibited molluscacidal activity against other genera of snails (Berry, Nolan and Gonzalez—1950; McMullen—1951) were obtained. Where possible, related compounds in the same chemical series were used. The method of exposing snails to the test chemicals was essentially the same as that used in the preceding paper. Aqueous dilutions of the test compounds were made in beakers containing a total volume of 500 cc. from 0.1 per cent stock solutions. The beakers were then agitated to obtain an even distribution. Five lymnaeid snails, of the species named above, were added to each test solution and to the paired control beaker containing ditch water. Observations were made at the end of 2, 4, 8 and 24-hour periods. After a 24-hour exposure the snails were removed from the test solution and placed in fresh water, and the mortality was recorded after 24 hours in fresh water. There was no mortality of any snail used as a control. In this series a total of 98 compounds were tested in concentrations varying from 1 : 100,000 to 1 : 1,200,000 (10–.833 ppm), based on active ingredients.

To check the effect of some compounds on liver fluke ova, fresh fluke ova were secured from bile of infected animals. After a six-day incubation period, the ova were placed in dilutions of test compounds for 24 hours, after which the test compounds were removed and fresh water was added. After the incubation period, a sample of ova was placed in tap water for a control. Daily observations were made as to the number of ova which hatched.

RESULTS

As indicated in Table 1, only three compounds had an LD 100 on the test snails

TABLE 1.—*Results of organic compounds tested as molluscacides*

A.	100% mortality in concentrations up to and including 1 : 1,200,000 (.833 ppm)
	1. Pentachlorophenol, 8%
	2. 2,4-dinitro-6-phenylphenol, 50%
B.	100% mortality in concentrations up to and including 1 : 1,000,000 (1 ppm)
	1. Pentachlorophenol, Tech.
	2. Dinitro-o-cyclohexylphenol, 38%

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TABLE 1—*Continued*

C. 100% mortality in concentrations up to and including 1 : 800,000 (1.25 ppm)
1. 2,4-dinitro-6-phenylphenol, Tech.
2. Bis(3-bromo-5-chloro-2-hydroxyphenyl)methane, 20%
3. Pentachlorophenol, 20%

D. 100% mortality in concentrations up to and including 1 : 600,000 (1.66 ppm)
1. Bis(3,5,6-trichloro-2-hydroxyphenyl)methane, Sodium Salt, 25%
2. P-diisobutylphenol, Tech.
3. X-x-diethyl-2-hydoxydiphenyl, Tech.
4. Bis(trichloro-hydroxyphenyl)methane, 20%
5. Copper pentachlorophenate, 60%

E. 100% mortality in concentrations up to and including 1 : 400,000 (2.5 ppm)
1. Sodium 2,4,5-trichlorophenate, 85%
2. 2,4,6-trichloro-3,5-xylenol, Tech.
3. O-propenyl-p-cresol, Tech.
4. 2,4,6-triisopropylphenol, Tech.
5. Bis(3,5-dichloro-2-hydroxyphenol)methane, 20%
6. 2-butoxyethyl *o*-chlorophenyl phenylphosphate, Tech.

F. 100% mortality in concentrations up to and including 1 : 200,000 (5 ppm)
1. O-cyclohexylphenol, Tech.
2. 2,4,6-trichlorophenol, Tech.
3. P-cyclohexylphenol, Tech.
4. Bis(5-chloro-2-hydroxyphenyl) sulfide, Sodium Salt, 25%
5. 2,2-methylenebis (4-chlorophenol), 60%
6. 4-tert-amyl-2,6-dichlorophenol, Tech.
7. 4-chloro-2-octylphenol, Tech.
8. P-bornylphenol, Tech.
9. Chlorocarvacrol, Tech.
10. P-tert-amyl-o-cresol, Tech.
11. 2,4-diamylphenol, Tech.
12. 2-chloro-4-phenylphenol, Tech.
13. 2-chloro-2-phenylphenol, Tech.
14. X-amyl-2-hydroxydiphenyl, Tech.
15. Tetraethyl monothionopyrophosphate, Tech.
16. Di-(2,chloroethyl) *p*-nitrophenyl thionophosphate, Tech.
17. Diethyl *o*-nitrophenyl thionophosphate, Tech.

G. 100% mortality in concentrations up to and including 1 : 100,000 (10 ppm)
1. 4 cyclohexyl-2,6-dinitrophenol, Tech.
2. 2,4-dichlorophenol, Tech.
3. P,p′-isopropylidenediphenol, Tech.
4. Bis(5-chloro-2-hydroxyphenol methane, Monosodium Salt, 40%
5. 2,4,5-trichlorophenol, Tech.
6. O-nitroso-m-cresol, Tech.
7. 4,6-dibromo-o-cresol, Tech.
8. P-tert-butylphenol, Tech.
9. 4-tert-butyl-2-chlorophenol, Tech.
10. O-amylphenol, Tech.
11. P-tert-amylphenol, Tech.
12. 4-tert-amyl-2-chlorophenol, Tech.
13. 4-chloro-3,5-xylenol, Tech.
14. O-allyl-p-cresol, Tech.
15. 4-chloro-2-phenylphenol, Tech.
16. O-benzylphenol, Tech.
17. Alpha-phenyl-p-cresol, Tech.
18. N,N-Amylcyclohexylbenzylamine, Tech.
19. *ω*-Chloro-*o*-nitrobiphenyl, Tech.
20. Ethyl bis(*p*-chlorophenyl)phosphinate, Tech.
21. Tetrapropyl monothionopyrophosphate, Tech.
22. Tetraethyl dithionopyrophosphate, Tech.
23. Tetraisopropyl monothionopyrophosphate, Tech.
24. *p*-Nitrophenyl dipropyl thionophosphate, Tech.
25. Ethyl methyl *p*-nitrophenyl thionophosphate, Tech.
26. 1,1-Bis(*p*-chlorophenylmercapto)ethane, Tech.
27. *p*-Benzyl phenol, Tech.
28. Heptanoyl chlororesorcinol, Tech.
29. Chloro-aceto-hexanoyl-resorcinol, Tech.
30. *n*-Butyl chlororesorcinol, Tech.
31. *n*-Butyl phenol, Tech.

H. Not 100% mortality in concentrations of 1 : 100,000 (10 ppm)
1. P-sec butylphenol, Tech.
2. P-tert butylphenol, Tech.
3. Sodium o-phenylphenate, Tech.
4. Sodium salicylate, Tech.
5. 3-phenylsalicylic acid, Tech.
6. Sodium 1-phenol-4-sulfonate, Tech.
7. Beta-chloro-ethyl decyl, Sulfite
8. 2,4,5-trichloro-phenoxyacetic acid, 37%
9. P-chlorophenate, Sodium Salt, 25%
10. 2,4-dichlorophenate, Sodium Salt, 25%
11. Sodium trichloroacetate, 90%
12. 2,4-dichlorophenoxyacetic acid, Sodium Salt, Tech.
13. 2,4,5-trichlorophenoxyacetic acid, Sodium Salt, Tech.
14. Trichlorobenzene, 96%
15. 1,2-dichloro-x-nitrobenzols, 88%
16. 2-chloro-4,6-dinitrophenol, 50%
17. O-nitrophenol, Sodium Salt, Tech.
18. O-chlorophenol, Tech.
19. 4-chloro-m-cresol, Tech.
20. 2,5-xylenol, Tech.
21. O-propyl-p-cresol, Tech.
22. Thymol, Tech.

TABLE 1—*Continued*

23. Isothymol, Tech.
24. Carvacrol, Tech.
25. 2,chloro-4,6-ditert-amylphenol, Tech.
26. Tri-terposol No. 8 Borate, Tech.
27. O-chloro-benzaldehyde 1–3,glycerin acetal, Tech.
28. Dypone, Tech.
29. Chlorinated p-cresol, Tech.
30. Tetrabromothiophene, Tech.
31. 3,4-Dichloro-a-methylbenzyl alcohol
32. Hexadecyl resorcinol, Tech.

in 24 hours at concentrations of 1 ppm or less. Pentachlorophenol, technical, dissolved in alcohol and as a commercial product diluted in kerosene, gave high mortalities. A mixture of 2,4-dinitro-6-phenylphenol and wetting agents gave higher mortality than did the technical product dissolved in alcohol. Neither alcohol nor kerosene in concentrations of 5 ppm had any molluscacidal activity. A new formulation of dinitro-o-cyclohexylphenol was very toxic and irritating to the snails, causing many to leave their shells. The bromination of bis(3,5-dichloro-2-hydroxyphenol)methane doubled its molluscacidal activity. Apparently the toxicity of the chlorophenol compounds is directly proportional to the degree of chlorination of the phenol radical. The addition of sodium or copper salts to pentachlorophenol decreased its molluscacidal activity.

Fluke ova in the control sample hatched in 9–14 days at room temperature. Following a 24-hour exposure in the laboratory to sodium pentachlorophenate in concentrations of 2.5 ppm, no ova hatched during 47 days, as indicated in Table 2.

TABLE 2.—*Results of organic compounds tested as ovocides*

Compound	PPM	No. Ova	Number of Ova Hatched During a 47 Day Period
Dinitro-o-cyclohexylphenol, Dicyclohexylamine Salt	10	65	None
	5	30	None
	2.5	1425	1401
Dinitro-o-cyclohexylphenol	10	620	33
	5	985	251
	2.5	95	91
Sodium Pentachlorophenate	10	133	None
	5	141	None
	2.5	153	None
Sodium 2,3,4,6-Tetrachlorophenate	10	353	341
	5	114	112
	2.5	36	35
4 Cyclohexyl-2,6-dinitrophenol	10	231	195
	5	201	185
	2.5	155	142
2,4-Dichlorophenol	10	61	56
	5	27	25
	2.5	216	216
Sodium 2,4,5-Trichlorophenate	10	128	None
	5	31	None
	2.5	110	26
P-Sec. Butylphenol	10	193	184
	5	215	213
	2.5	86	85

All ova failed to hatch after exposure to concentrations of 5 ppm of dinitro-o-cyclohexylphenol, dicyclohexylamine salt, but did hatch following exposure to 2.5 ppm. Sodium 2,4,5-trichlorophenate in concentrations of 5 ppm inhibited the hatching of ova but failed to do so in concentrations of 2.5 ppm.

SUMMARY

1. A total of 98 compounds were evaluated for their molluscacidal activity against lymnaeid snails. Pentachlorophenol, 2,4-dinitro-6-phenylphenol and dinitro-o-cyclohexylphenol gave 100 per cent mortality following 24-hour exposure in concentrations of 1 ppm. These compounds show the most promise as molluscacides and will be tested under field conditions.

2. Fluke ova failed to hatch after an exposure of 24 hours to 2.5 ppm of sodium pentachlorophenate, 5 ppm of dinitro-o-cyclohexylphenol, dicyclohexylamine salt, or 5 ppm of sodium 2,4,5-trichlorophenate.

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THELANDROS ALATUS WEDL, 1862 (NEMATODA: OXYURIDAE) AND ITS SYNONYMS

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Thelandros alatus is an oxyurid, or pinworm, of lizards. According to Chitwood's (1937) division of the Oxyuridae, it falls, along with other so-called one-spiculed oxyurids from reptiles, in the subfamily Pharyngodoninae; the remaining subfamily, the Oxyurinae, recognized by Chitwood, includes the one-spiculed pinworms from mammals.

T. alatus is the type of *Thelandros* which is antedated by only one pharyngodonin genus. Although this species has been known for 90 years, consensus has not been attained as to some features of the male morphology.

The main points at issue are whether this male is provided with lateral alae, or a caudal prepuce, or both. The question whether the presence or absence of a precaudal cuticular inflation, or caudal prepuce, is of taxonomic value also is involved. Upon the actual facts in these respects and upon the answer to this question, depend not only the generic characters, strictly speaking, but also to a large extent, if not entirely, the status of certain other presently recognized *Thelandros* species.

From a careful review of the descriptions appertaining to this genotype and to the other species in question and from a study of certain specimens in the U. S. National Museum Helminthological Collection, the following conclusions have been reached.

(1) *T. alatus* applies to a species in which the male has the following characteristics not universally attributed to it: (a) the body completely lacks lateral alae; (b) in preserved specimens, an area of the precaudal cuticle adjacent to the tail, but somewhat variable in anterior extent, usually is inflated; (c) in some such specimens the inflation projects primarily dorsally and posteriorly and envelops the anterior part of the tail, including part of the dorsal spike, i.e., a "caudal prepuce" is present; (d) the two large adanal laterodorsal genital peduncles or processes may appear more or less rectangular in shape when viewed laterally, but actually are distally bilobed; (e) these processes are mono-papillate; the ventral lobe of each bears a papilla which is surrounded by a circlet of cuticular elevations; the dorsal lobe contains "pulp" and possibly nerve fibres; it is drawn out to a point distally, creating the impression that it may bear a papilla at this point, but there is no external evidence of the presence of a papilla there (Fig. 1); (f) the anterior anal lip is echinate (Fig. 2); (g) the large fleshy, bluntly conical, dorsal anal lip bears subventrally a pair of small papillae at or near its distal tip; (h) when this lip is viewed laterally, its border, particularly dorsally, appears to be reinforced internally by hyaline material; when viewed ventrally, hyaline bars appear to be present internally along its lateral margins, i.e., a V-shaped supporting structure appears to be present (Fig. 3); light refraction from the convex cuticular surfaces of this conical projection perhaps may be responsible for these appearances.

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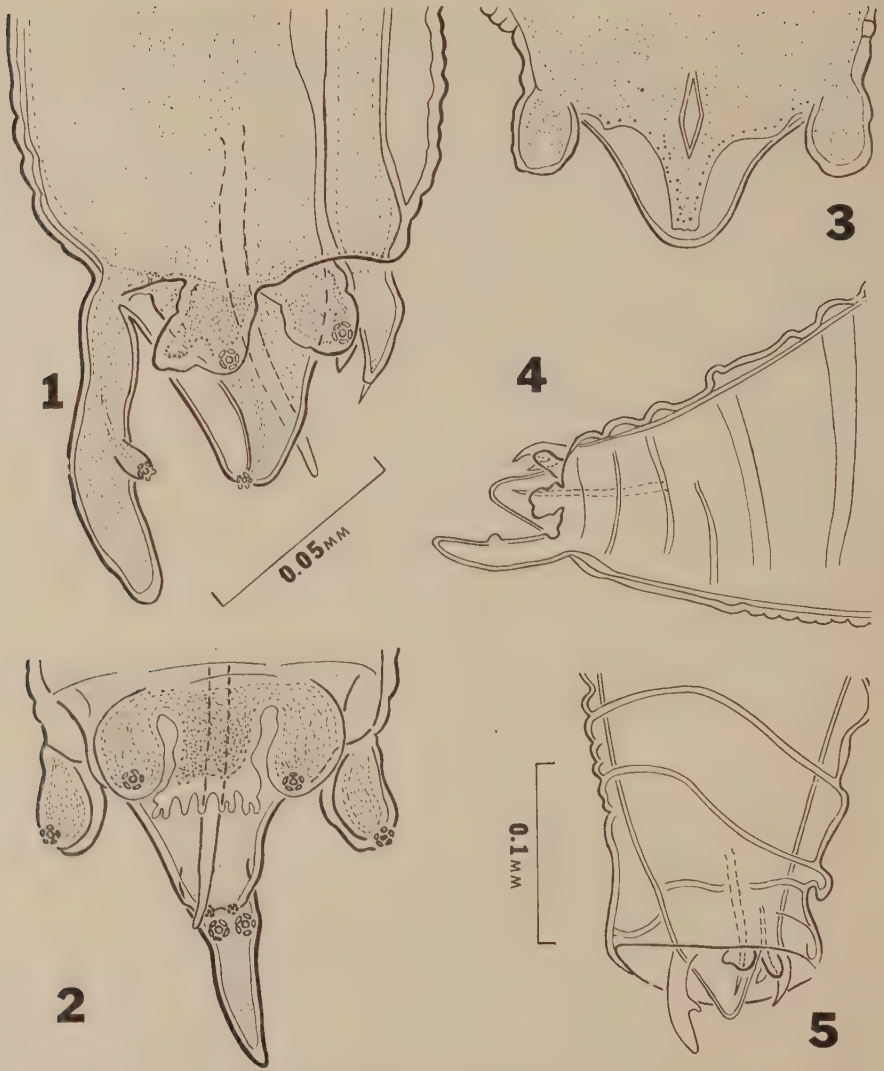

FIGS. 1–5.—*T. alatus* Wedl: 1, Genital and precaudal region in lateral aspect (U. S. N. M. No. 47078); 2, genital region in ventral aspect (same specimen); 3, optical longitudinal, frontal section through dorsal anal lip (and stalks of sublateral papillae), showing hyaline appearance of its laterodorsal margins (same specimen); 4, outline of genital and precaudal regions in lateral aspect to show slight, discontinuous separation of cuticle ventrally and laterally (U. S. N. M. No. 27503; originally determined as *T. "sahariensis"*); 5, outline of genital and precaudal region in lateral aspect to show precaudal cuticle inflated to form a "caudal prepuce" (U. S. N. M. No. 27503). Figs. 2 and 3 drawn to same scale as Fig. 1; Fig. 4 to same scale as Fig. 5).

(2) *T. micruris, T. sahariensis* and *T. avis* are synonyms of *T. alatus* and the suppression of these names is hereby proposed.

These conclusions rest upon the following observations and interpretations:

The evidence of the original and later descriptions of T. alatus

Wedl (1862) stated that the male of *T. alatus* has in the genital region of the body two broad lateral cuticular wings which terminate in front of the posterior end of the worm. However, his illustrations show that his specimens actually had in this region not lateral alae, but a precaudal cuticular inflation extending posteriorly about to the level of the origin of the dorsal caudal spike. Similarly, Caleb (1889) stated that in the male of "*Oxyrus Uromasticolla*," which generally is considered a synonym of *T. alatus*, the cuticle at the posterior extremity is expanded giving rise to two lateral alae. However, his figure of the male tail region shows with reasonable certainty that a caudal prepuce was present in actuality.

Seurat has redescribed the genotype three times, twice (1912; 1917) under the name "*Thelandros alatus*" and once (1915) under the name "*Oxyuris uromasticola*." In the last-mentioned description, he (1915) stated that by examining living worms he had verified that the lateral alae described by Wedl do not in reality exist and added the observation that preservation of specimens in alcohol frequently produces a detachment of the cuticle which Wedl wrongly interpreted as an ala. In his final redescription, he (1917) reemphasized that the species lacks lateral alae, but he did not repeat his observations on the cuticle of the male, evidently because he did not consider the inflation produced by preservation a true specific attribute. Since this redescription evidently is the one which has been consulted mainly by those who subsequently have dealt with the species, this omission appears to be responsible for the misconceptions held by some subsequent authors.

The last redescription to appear under the name "*Thelandros alatus*" was published in 1925 by Thapar, who stated that the cuticle bears lateral alae "along its entire length," but clearly figured a pronounced ventral precaudal cuticular inflation in the male.

Wedl observed only one of the two sublateral genital processes actually present, but described it as distally bifid. Seurat (1917) observed both of them and noted that each bears a papilla. Thapar's (1925) illustration (Fig. 83) evinces a possible intention to figure them as bi papillate. But he described these "pedunculated papillae" as "forked at the tip," rather than double. Wedl stated that one of the genital processes, identifiable beyond doubt as the anterior anal lip, bears a hooklet. Seurat (1917) described this process as dentate. Thapar stated that it is simple. Wedl depicted two small prominences at the distal tip of the dorsal anal lip. Seurat (1917) stated that this truncate appendage bears a pair of papillae at its extremity. Thapar did not mention these papillae. He stated: "An accessory piece is absent but in a few specimens its position is indicated by a triangular and hyaline structure." Seurat (1915) stated that a "gorgoret" is absent, but in his (1917; Fig. 5, B.) figure of the tail in a ventral aspect are shown, just within the lateral margins of the dorsal anal lip, two darkly stippled bars evidently intended to represent supporting structures.

The "differences" between T. micruris, T. sahariensis and T. alatus

T. micruris Rauther, 1918 was originally proposed, as far as Rauther (1918) stated, simply because the specimens upon which it is based were believed to differ from *T. alatus* in size and proportions; his specimens were smaller than Wedl's.

T. sahariensis Baylis, 1930 was proposed because the specimens upon which it

is based were considered to differ from Rauther's in size sufficiently to make their identification as *T. micruris* questionable; Baylis' specimens were smaller than Rauther's.

However, Baylis (1930) recognized *T. micruris* and proposed *T. sahariensis* under the misapprehension that in having a caudal prepuce the males upon which these species are based differ from *T. alatus* males. He mentioned specifically no other differential feature and the fact is that the descriptions of the two species in question agree with Seurat's (1917) final redescription of *T. alatus* in all other morphologic essentials. Hence, these three descriptions become fully equivalent, when Seurat's is interpreted in the light of his (1915) previously recorded statement that the cuticle is usually inflated in preserved males. Moreover, Wedl's original description, although it contains a few misinterpretations, is fully reconcilable with the descriptions given by Rauther and Baylis. Rauther (1918) described the sublateral adanal papillae as double, whereas Baylis (1930) and, as previously noted, Seurat (1917) observed a single papilla on each adanal process, but this difference undoubtedly is subjective for, even on careful study, it is very difficult to determine whether these processes are mono- or bi-papillate. Neither Rauther nor Baylis noted a supporting structure within the dorsal anal lip, but, as previously stated, the impression that such a structure is present may be due to an optical illusion.

Writer's observations; opinions concerning "caudal prepuce"

The Museum Collection contains two males identified as *T. alatus.* One (No. 29213) is from *Uromastix* sp. and the other (No. 47078) from *U. spinipes,* the type host of Wedl's species; both hosts died at the National Zoological Park, Washington, D. C. The precaudal cuticle is noticeably inflated, more so ventrally than dorsally or laterally, in the first specimen. The inflation is less pronounced in the second; both have all of the other characteristics enumerated for the species earlier in this note.

This Collection also contains 21 males identified as *"T. sahariensis"* (No. 27503); the host was a *Uromastix* sp. of African origin and died soon after its arrival at the National Zoological Park. They, of course, are regarded by the writer as *T. alatus* and in this rather small series of specimens from an individual lizard the following variations were noted: precaudal cuticle elevated into annular folds, but not forming a continuous inflation (2 specimens; Fig. 4); cuticle of this area inflated but not enveloping the tail (13 specimens); cuticle more extensively inflated, but barely enveloping the dorsal caudal spike near its origin (4 specimens); a well-developed "caudal prepuce" present (2 specimens; Fig. 5).

Since even on the criterion of size, these males (length, about 3.5–4.5) are *T. alatus,* these observations confirm the evidence found in the literature, and derivable from its interpretation, and show the supposition to be false that *T. micruris* and *T. sahariensis* are distinguishable from *T. alatus* by the presence of a caudal prepuce.

As to the cause of the inflation, Seurat (1915) is not alone in considering fixation, or factors closely related to this, responsible. Chatterji (1935) characterized the male of *T. baylisi* as having the "posterior end" enclosed in a prepuce-like sheath; he illustrated the "sheath" as precaudal and as markedly annular. He stated that in the few specimens in which the cuticular separation was very pronounced, resembling the inflation illustrated for *T. micruris* and *T. sahariensis,* it obviously was due to

plasmolysis; he suspected that this explanation likewise applied to these two "species." Pereira (1937) drew attention to the extensive variation observed by him in the "contractility" of the male tail of *T. sceleratus* Travassos, 1923, a prepuce-like formation being present in some specimens and apparently absent in others, and remarked that such variability may lead to species misidentifications.

The Museum specimens, as well as many of those studied by other observers, undoubtedly were not fixed until after the hosts had been dead for a considerable time. Perhaps the effect most frequently caused by the partial disintegration of nematodes is the separation of the cuticula or cuticular layers. It is a striking fact, however, that none of the mentioned observers has noted cuticular inflation in the female worms or along any part of the male body, except near the tail Whatever the causation, it is quite clear that preserved males of the same species may have no, or virtually no, inflation or a definite caudal prepuce. The absence, presence, or extent of the inflation, therefore, cannot be regarded as a specific character in the usual sense. However, it appears to the writer that mention of a tendency toward cuticular inflation quite properly may be included in the description of those species known to exhibit this phenomenon.

Separation of T. alatus, T. micruris and T. sahariensis on size not justified

The specimens in the Museum Collection do not offer evidence that *T. alatus* is markedly variable in size. Nevertheless, the writer believes that there are not adequate grounds for the separation of *T. alatus*, *T. micruris* and *T. sahariensis* on the basis of large, medium and small size, respectively.

The size range reported for *T. alatus* by Seurat (1917) includes Rauther's measurements for *T. micruris*, as interpreted, no doubt correctly, by Baylis (1930; 1936). Moreover, the maximum length given for the male of *T. sahariensis* is only 0.07 mm. less than the minimum length given by Seurat for *T. alatus*. The males before Seurat and Baylis, therefore, varied almost continuously in length from 2.2–4.6 mm.; to consider two morphologically indistinguishable males different species because one is 2.43 mm. and the other 2.5 mm. in length seems to the writer an exceedingly arbitrary action. These authors reported very nearly the same length for the spicule; Rauther did not report its length. There are reported differences in egg size and the size and proportions of certain organs among the specimens studied by these observers; the longest of Baylis' females was 0.8 mm. smaller than the shortest of Seurat's. But the significance of these differences is questionable. Different observers rarely obtain identical measurements even from the very same specimens.

T. avis Maplestone as a synonym of T. alatus

Maplestone (1940) stated that *T. avis* shows sufficient differences from all previously proposed species to justify its recognition as distinct, but did not specify their nature or compare his specimens with any particular species. In order to consider Maplestone's description applicable to *T. alatus* it is necessary to assume only that his statement that the ventral anal lip is "not fringed" is incorrect and that he overlooked the presence of papillae on the opposite lip. These assumptions are considered justifiable. All that is known as to the hostal occurrence of pharyngodonin oxyurids favors the view that his specimens, which are "said to have been found . . . in . . . a sandpiper . . ." actually must have been of reptilian origin.

Maplestone's figure of the male tail suggests that he considered the sublateral, and possibly even the subventral, papillae to be duplex. He stated that there "are two pairs of large papillae on each side of the cloaca," but this statement evidently is not to be taken literally, since he further stated that there is "a third pair" of papillae on the dorsal caudal spike. As a matter of fact, in *T. alatus* males the mono-papillate, subventral, genital processes tend to be slightly bilobular distally, though not so markedly so as the sublateral ones, or as Thapar's (1925) figure suggests.

The identity of Thapar's specimens

If Thapar correctly described his specimens, he did not have before him any of the species[1] which are based on worms from *Uromastix*. But the writer believes that Thapar's male specimens definitely were *T. alatus,* since his (1925) figure of the tail region is so consistent with this identification. In order to regard his description as applicable to this species, it must be assumed to be erroneous in the same respects as Maplestone's and also in its insistence upon the presence of lateral alae. Baylis evidently assumed that it contains these errors, since he definitely synonymized *T. alatus* of Thapar with *T. micruris*. With the writer's suppression of *T. micruris,* Thapar's original identification is automatically upheld.

ABSTRACT SUMMARY

The morphology of the genital region of the male of *T. alatus* is redescribed because some subsequent authors have not interpreted the original description correctly and have misunderstood Seurat's correct concept of the species. These misapprehensions have led to the false supposition that the males on which *T. micruris* Rauther and *T. sahariensis* Baylis are based differ morphologically from the male of *T. alatus*. Since it also seems illogical to separate these males specifically on the basis of size, the suppression of *T. micruris* and *T. sahariensis* as synonyms of *T. alatus* is proposed. Since the description of *T. avis* Maplestone agrees with *T. alatus* in all but two respects, in which it is assumed to be erroneous, the suppression of this name as synonymous with the genotype also is proposed.

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[1] Seven "species" have been described from lizards of this genus: *T. alatus, "Oxyrus Uromasticolla"* and *T. saharicnsis* are based on worms from North African *Uromastix,* whereas *T. micruris, T. kausali, T. baylisi* and *T. taylori* are based on specimens from the Indian *U. hardwickii,* the host of Thapar's material. By the actions of the writer and previous authors, this number has been reduced to four, *T. alatus* and the three last-mentioned species.

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SUSCEPTIBILITY OF RICE RATS *(ORYZOMYS PALUSTRIS)* TO *SCHISTOSOMA MANSONI*[1]

DONALD V. MOORE AND HENRY E. MELENEY

Schistosoma mansoni is essentially a human parasite and although Cameron (1928) found monkeys naturally infected with this parasite there are no records of any other animal serving as a true reservoir host. Common laboratory animals can be successfully infected, and the suitability of laboratory animals as experimental hosts has been recently studied by Moore *et al.* (1949) and Stirewalt *et al.* (1951). Stirewalt *et al.* also reported the successful infection of the cotton rat with *S. mansoni.* Although the cotton rat is frequently used as an experimental animal in the laboratory the species does not represent a highly inbred strain of animal and may still be considered as a wild rodent.

MATERIALS AND METHODS

Mr. William Hegener of Sarasota, Florida, called our attention to the semi-aquatic habits of the rice rat (*Oryzomys palustris*). Several specimens of the rice rat escaped from their cages in his laboratory and ravished several aquaria in order to obtain aquatic plants and snails, ignoring the regular animal food which was present in abundance. He found that these animals would enter the water in the aquaria in order to obtain the preferred food.

The semi-aquatic habitat, preference for aquatic foods, and willingness to enter water to obtain food led to the speculation as to whether or not these animals would be susceptible to *S. mansoni.* Through the cooperation of Mr. Hegener we obtained several rice rats for experimental use. Two sub-species were received: *Oryzomys palustris palustris* and *O. palustris natator.* No attempt was made to separate the sub-species in the evaluation of the results.

In order to simulate natural conditions of exposure the hair of the animals was not clipped prior to exposure. The animals were kept in small individual cages and it was found that when these cages were placed on end in water they made no attempt to climb out of the water, but actually seemed to prefer to sit in the water. This served as a convenience in the immersion of the animals in cercaria-infested water. Eleven of the animals were exposed to 150 cercariae each and 11 were exposed to 250 cercariae each. Stool examinations by direct smear and the sedimentation-hatching technique were begun five weeks after exposure and continued on a weekly basis until eggs were demonstrated. Thereafter stool examinations were done every other week until forty-one weeks after exposure after which time no more stool examinations were done. The animals were sacrificed between thirty-two and forty-eight weeks after exposure. The schistosomes were recovered by the perfusion technique of Yolles *et al.* (1947). At the time the animals were sacrificed a search was made for other helminths naturally harbored by the rice rats. The helminths so obtained will not be discussed in the present paper.

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[1] From the Department of Preventive Medicine, College of Medicine, New York University. This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

RESULTS

The tabulation of the schistosome recovery is shown in Table 1. It will be noted that in both groups there was an excess of male worms. In Group A (150 cercariae each) 82.6 per cent of the total worms recovered were males, in Group B (250 cercariae each) 66.0 per cent were males. One negative animal was found in each group and one half of the animals harbored only male worms. The cercariae used for infection were pooled from several snails but obviously there was a predominance of male infections in the snails. The negative animals and those harboring only male worms are all included in the tabulation of the results.

Considering both groups together with regard to the distribution of the worms between the intra-hepatic and mesenteric portions of the portal circulation we find that 61.3 per cent of the worms were in the liver and 38.7 per cent in the mesenteric circulation. This distribution may have been due to the fact that these animals harbored an excess of male worms, since relatively few unpaired males migrate to the mesenteric vessels. This distribution seen in the rice rat compares favorably with that in the hamster as reported by Moore *et al.* (1949) where 59 per cent of the worms were found in the liver 12 weeks after infection. In mice on the other

TABLE 1.—*Recovery and distribution of* S. mansoni *in rice rats*

Group	Number cercariae per animal	No. of animals used	Total cerc. used	Worms recovered						Distribution of worms				Eggs first found in feces
										Liver		Mes.		
				No. of worms	% Rec.	No. ♂	% ♂	No. ♀	% ♀	No.	%	No.	%	
A	150	11	1650	46	2.79	38	82.6	8	17.4	30	65.2	16	34.8	9 wks.
B	250	11	2750	47	1.71	31	66.0	16	34.0	27	57.4	20	42.6	7 wks.

hand Moore *et al.* found that 12 weeks after infection 38 per cent of the worms were from the liver. Both mice and hamsters are considered as satisfactory hosts for *S. mansoni.*

The percentage worm recovery in each group was low; 2.79 per cent in the group exposed to 150 cercariae each and 1.71 per cent in the group exposed to 250 cercariae each. This recovery is much lower than the expected worm recovery for mice and hamsters, but is comparable to the expected recovery in albino rats. However, in albino rats the worms are confined to the liver, egg lesions are not found in the intestinal wall, nor are eggs passed in the feces, Moore *et al.* (1949).

As stated previously only one half of the 22 animals developed double sex infections. Of the 11 animals with a double sex infection, the number of animals with eggs in the various tissues as detected by press preparations was as follows: lungs 3, liver 11, spleen 4, duodenum 9, jejunum 11, ileum 10, and colon 7. Since these were all old infections many of the eggs in the tissues were calcified; however, viable eggs were found in each animal.

The egg lesions in the lungs appeared grossly as small amber-colored raised areas on the pleural surface. When eggs were present in the liver the surface of the liver presented a roughened appearance and numerous grayish-white spots could be seen. The egg lesions in the intestines were easily detected grossly through the relatively thin wall. They appeared as whitish-brown streaks representing groups of eggs. The spleen was only slightly enlarged even in the animals harbor-

ing the most worms and having egg lesions in the spleen. Tissues from one animal were examined after sectioning. The histopathology seen was similar to that seen in albino mice infected with *S. mansoni.*

The appearance of viable eggs in the stools of the infected animals was first observed nine weeks after exposure in the animals given 150 cercariae each, and seven weeks after exposure in the animals receiving 250 cercariae each. Both groups were still passing viable eggs forty-one weeks after exposure, although at this time the miracidia were not as numerous as they had been earlier in the infection.

DISCUSSION

The report of Stirewalt *et al.* (1951) that the cotton rat is susceptible to infection with *S. mansoni* raised the question as to whether small rodents could serve as reservoir hosts for this parasite. According to Anthony (1928) the natural habitat of the cotton rat is grasslands, and the natural food consists of stems, foliage, seeds of plants, grasses, meadow growths, and cultivated crops. Worth (1950) found cotton rats in the dry uplands but also found them to be quite abundant in the wet lowlands in Florida. The presence of the cotton rat in wet lowlands undoubtedly depends on the sub-species and local conditions. We have also successfully infected the cotton rat with *S. mansoni* in our laboratory, and found that when they were placed in water for exposure they became excited and made every effort to climb out of the water.

According to Anthony (1928) the rice rat lives in grassy localities, marsh meadows, or open brush lands. Worth (1950) found that these rice rats trapped in his study were confined to the wet lowlands. The food of the rice rat consists of a variety of sedges, grasses, foliage of shrubs, seeds of native plants, and some animal food such as small crustaceans and mollusks.

In general the rice rat seems to be better adapted than the cotton rat to life near water. As stated previously, the rice rats used in this experiment made no attempt to climb out of the water when they were exposed to cercariae; in fact, they seemed to prefer to remain in the water. It is interesting to note that the distribution of some sub-species of the rice rat overlaps that of *Tropicorbis sp.* which has been experimentally infected with *S. mansoni.*

Since laboratory animals such as mice and hamsters can be easily infected with *S. mansoni,* and at least two species of wild rodents, the cotton rat and the rice rat, may also be successfully infected with this parasite, it seems not unlikely that a reservoir host for *S. mansoni* might exist or be developed.

SUMMARY

Rice rats, *Oryzomys palustris palustris* and *O. palustris natator,* have been successfully infected with *S. mansoni.* Although the worm recovery was low the schistosomes reached maturity and viable eggs were passed in the feces of the infected animals.

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INFECTIONS OF *ANCYLOSTOMA CANINUM* IN ABNORMAL HOSTS*

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It is of interest to ascertain the fate of the larvae of certain skin penetrating nematodes in abnormal hosts. If the abnormal host is closely related to the normal one, sometimes infection of a low order may be established. Such was the case of *Nippostrongylus muris* in the cotton rat and hamster as shown by Lindquist (1950). The fate of the larvae appears to differ in details depending probably on both host and parasite. There is little doubt that those nematode larvae entering the blood stream may be encountered in a variety of different places in both the normal and abnormal hosts. In the earlier study the retention of the larvae of *N. muris* was reported in the skin and lungs of the cotton rat and they were also seen in the mesenteries and kidneys (Lindquist, 1950). Scott (1928) mentioned finding *Ancylostoma caninum* larvae in both liver and lungs of the laboratory rat 21 days after infection. Spindler (1943) found *Strongyloides ratti* distributed throughout the body organs of the normal host. He also showed that the larvae of *Strongyloides ransomi* in swine may enter the heart musculature causing great damage and even death.

Infection experiments were carried out with *Ancylostoma caninum* in the laboratory and cotton rat to see whether or not there was a retention of larvae in these host-parasite combinations. Only the skin and lungs were examined for comparison with the previous work on *Nippostrongylus muris* (Lindquist, 1950). The larvae of the dog hookworm were obtained from a stock strain kept in the Parasitology Department of The Johns Hopkins University School of Hygiene over a period of years. The handling of skin infections, hosts and the sectioning of tissue was related in detail in the earlier paper.

HISTOPATHOLOGY IN THE SKIN AND LUNGS OF THE LABORATORY RAT

Skin. A young animal, infected by skin penetration with 5,125 infective larvae of *A. caninum* was autopsied at 26 hours and sections vertical to the surface of the skin were made. Eight out of 30 slides with about 300 sections showed larvae trapped in nodules in the reticular layer of the derma. The cellular formation around them was the same as reported for *Nippostrongylus muris* in the cotton rat. The larvae, retained in the cell masses, were stained well with haemotoxylin and did not seem to have undergone any disintegration at this time. (Fig. 1.)

Lung. Most of a lung lobe of the same animal in which nodules were found in the skin was sectioned. Sixty-one serial slides were made but only 3 of them showed larvae. They were found free in the alveolar spaces, but there did seem to be a hyperactivity of inflammatory cells.

Part of a lung lobe of another young laboratory rat, that had a 26 hour infection of 1,590 larvae of *A. caninum* by subcutaneous route was made into slides. In examining 25 slides, 14 of which showed larvae, no developing nodules were found (Fig. 2). The over-all picture of the lung was one of extensive damage with a

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* Contribution from the Department of Parasitology of the School of Hygiene and Public Health of the Johns Hopkins University.

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general mobilization of wandering cells and leucocytes scattered throughout the sections, plus large areas of hemorrhage and generally thickened alveolar septae.

One young rat, given 10,000 larvae by subcutaneous route, appeared so ill that it was sacrificed at 21 hours. Eighteen of the 20 slides that were made of lung tissue showed larvae. The lung damage was intense with profuse hemorrhage and general hyperactivity of the leucocytes and wandering cells, but there was no evidence of cellular encapsulation.

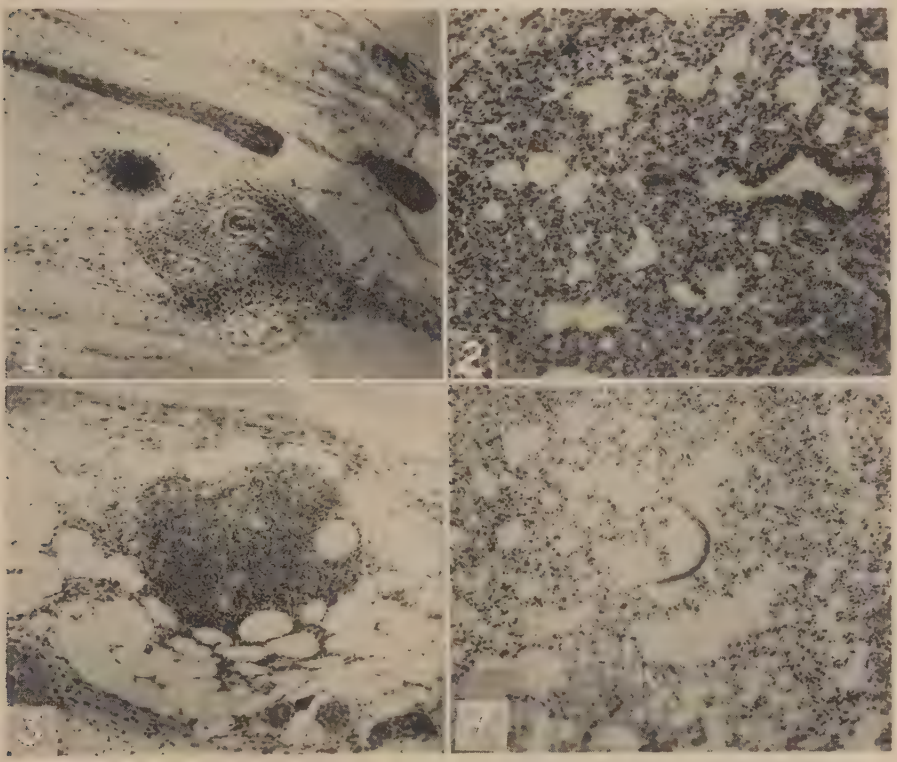

FIG. 1. Laboratory rat skin 26 hours after infection with *A. caninum*. 100×.

FIG. 2. Laboratory rat lung 26 hours after infection with *A. caninum*. 100×. Note the absence of cellular nodulation.

FIG. 3. Cotton rat skin 26 hours after infection with *A. caninum*. 100×.

FIG. 4. Cotton rat lung 24 hours after infection with *A. caninum*. 100×. Note the absence of cellular nodulation.

HISTOPATHOLOGY IN THE SKIN AND LUNGS OF THE COTTON RAT

Since it was shown that there was cellular encapsulation of the larvae of *A. caninum* in the skin of the laboratory rat but no retention in the lungs at 26 hours after infection, it was decided to see whether or not this same relationship held true in the cotton rat.

Skin. Using 5,125 larvae to infect a young cotton rat by skin penetration it was possible to demonstrate at 26 hours good nodular formation in one out of 15 slides made in serial section. The relationship of the cells to the larvae was typical of the encapsulation occurring in the laboratory rat (Fig. 3).

Lung. Fifteen slides were made from lung tissue taken at 24 hours from a young cotton rat that had 1,590 infective larvae injected subcutaneously. Two of these slides showed larvae but they were free in the tissue with no nodulation. The same picture of general damage was present in the laboratory rat lung (Fig. 4).

Thirteen slides made from lung tissue taken at 21 hours from a young cotton rat that had 10,000 larvae injected subcutaneously revealed great damage with large areas of hemorrhage and alveoli filled with fluid. Larvae were found in 12 of these slides, lying free in the alveoli, but there was no nodular formation.

The larvae of *Ancylostoma caninum* in the cotton rat appear to evoke the same tissue reaction as in the laboratory rat. There was nodular retention of larvae in the skin but not in the lung, which, however, showed considerable damage.

DISCUSSION

Although it has been pointed out that the infective larvae of *Nippostrongylus muris* are held up by cellular encapsulation in both the skin and lungs of cotton rats as early as 19 hours after infection (Lindquist, 1950), the same does not appear to hold true for *Ancylostoma caninum* in this host. The larvae of this species are encapsulated in the skin but lung encapsulation was not observed. The failure of the cotton rat to react toward nodulation in the lung phase is not easy to explain. Perhaps 26 hours after infection is too short a period for nodulation to start with *A. caninum* although it readily occurs with *N. muris* in the cotton rat. Further studies with sections at later intervals would be desirable in this respect. However, another possible explanation lies in the difference of the life cycles of the two nematodes involved. It is well known that *N. muris* stops to perform a molt in the lungs and also increases in size in this location (Yokogawa, 1922). During this lag the larvae may be caught by cellular infiltration. The larvae of *A. caninum,* which is similar to *A. duodenale* in life cycle, penetrate rather rapidly through the lung tissue without significant development. This more rapid passage of the hookworm larvae may account for the failure of the host's cells to retain them in nodules.

In the laboratory rat the same type of cellular reaction occurs with this parasite and here also there was no cellular attempt to isolate larvae in the lung by 26 hours after infection.

SUMMARY

Studies were made of cellular reaction to infective larvae of *A. caninum* in the lungs and skin of both cotton and laboratory rats.

The cellular reaction appeared the same in both hosts with encapsulation of the larvae in the skin but not in the lungs at 26 hours after infection.

Failure to get lung encapsulation may be due to a difference in the life cycle of *A. caninum* from that of *Nippostrongylus muris* which was shown in previous work to be encapsulated in the lung of the cotton rat.

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RESEARCH NOTES

ATTEMPTS TO EXCHANGE ASSOCIATED ORGANISMS IN CULTURES OF *ENDAMOEBA HISTOLYTICA*

Many unsuccessful attempts have been made to grow *E. histolytica* without an associated organism. Monobacterial associates were isolated by Dr. Rees and his group, and recently a *T. cruzi* associate without bacteria has been isolated by their microisolation technique (Phillips and Rees; Amer. J. Trop. Med., 1950). This laboratory has obtained from Dr. C. W. Rees (National Institutes of Health, Bethesda, Md.) ameba cultures of three types: ameba (N.I.H.-103)-"t," ameba-"streptobacillus," and ameba (F-22)-*T. cruzi*. Although the amebas are of different strains, it was thought that being able to isolate a single associate ameba culture at will would be desirable for metabolic studies and other work where strain specificity was not essential.

The first series of experiments were designed to obtain an ameba-trypanosome culture from either an ameba-"t" or an ameba-"streptobacillus" culture. The procedure involved placing into a centrifuge tube, 3 ml. of a trypanosome culture, 1 ml. of 0.3% Na thioglycollate, and 1 ml. of the sediment from a culture of either ameba-"t" or ameba-"streptobacillus" and then sealing with petrolatum in a fashion employed by Phillips and Rees (1950) and also by this laboratory. Before sealing, however, antibiotics were added to these cultures in order to eliminate the "t" and "streptobacillus." Streptomycin, 1:500, penicillin G, 1,000 units per tube, and bacitracin, 1:20,000 was added to each culture tube. These concentrations of antibiotic do not kill the ameba (Bradin and Hansen, Amer. J. Trop. Med., 30: 27–41, 1950), nor do they have any effect on the oxygen uptake of *T. cruzi* (unpublished data). These culture tubes were incubated at 37° C. and examined at 24-hour and 48-hour incubation. No ameba were detected when a drop of the sediment was removed and observed under the microscope. There was no evidence of bacterial growth. The trypanosomes remained viable. Subcultures were also negative for amebic growth. This result seems to indicate that the ameba could not be converted from a bacterial associate to a trypanosome associate under our experimental conditions.

The next work was performed in order to obtain a monobacterial associate from an ameba-trypanosome culture. Pure cultures of "t" and "streptobacillus" were obtained and grown on a liver-proteose-peptone medium that had been starched. Since our earlier work has indicated that the trypanosomes may be inactivated by heat-treating at 48° C. for 10 minutes, without killing the amoebae, the 48-hour cultures of the ameba-trypanosome were heat-treated and 1 ml. of the sediment inoculated into liver-proteose-peptone media. Ten tubes were inoculated with ameba which were simultaneously inoculated with "t" in one series and "streptobacillus" in another series. Another set of tubes were prepared by inoculating the amebae into a pre-conditioned medium where the bacteria had been growing for 24 hours. Also a similar set of inoculations were performed without heat-treating the ameba-trypanosome inoculatum. These tubes were all incubated at 37° C. and examined after 48 hours, 72 hours and 96 hours. No growth of ameba was detected in any of the tubes. Subcultures were also negative for amebic growth.

These experiments seem to indicate the difficulty with which amebae can go from one environment to another. Attempts to exchange associated organisms in cultures of *E. histolytica* were unsuccessful. It is difficult to speculate as to the reason for this failure to interchange the ameba cultures. Possibly it is due to the differences in the strains of the ameba involved.

This work was supported in part by the National Institutes of Health, Bethesda, Md.—Mitsuru Nakamura, *School of Medicine, University of California, San Francisco 22, California.*

NOTES ON HELMINTH PARASITES OF MUSKRATS FROM WESTERN COLORADO

In 34 muskrats (*Ondatra zibethicus osoyooensis* (Lord)) examined from Gunnison and Hinsdale counties, Colorado, between December 17, 1950 and April 29, 1951, the incidence of helminth parasites was as follows: trematodes: *Plagiorchis proximus* Barker in 26; *Notocotyle quinqueseriale* Barker and Laughlin in 26; *Echinostomum* sp. in one; cestode: *Hymenolepis evaginata* Barker and Noyes in four; nematode: *Trichurus opaca* Barker and Noyes in one. In addition, the mastigophoran *Giardia ondatrae* Travis occurred in several of the muskrats examined for protozoans.

The two localities are about seventy miles apart, on the western slope of the Rockies at altitudes of 7800 (Gunnison) and 8663 feet (Hinsdale Co.).

These preliminary findings indicate that a smaller number of species of helminth parasites may infect muskrats in this comparatively isolated region than in lower altitudes and latitudes

(cf. Barker, F. D., 1915. J. Parasit. 1: 184–197 and Chandler, Asa, 1914. J. Parasit. 27: 175–181.).—Lauren Ball, *Western State College, Gunnison, Colorado.*

HELMINTH PARASITES OF THE PETRALE SOLE[1]

During the summer of 1950 at Oregon Institute of Marine Biology, Charleston, Oregon, 213 petrale sole, *Eopsetta jordani* (Lockington) were examined for helminth parasites. A total of 174 trematodes identified as *Lecithochirium exodicum* (McFarlane, S. H., J. Biol. Bd. Canada 2: 335–347. 1936.), family Hemiuridae, were recovered from the stomachs of 59 of the fish. The incidence of infection was 28%. As many as 23 trematodes were recovered from a single host.

Larval encysted nematodes were recovered from all tissues of the fish. The nematodes belonged to the family Ascaridae, and probably either to the genus *Anisakis* or *Porrocaecum*. Due to their immaturity, generic characters were not sufficiently developed for diagnosis. From 91 of the fish examined 414 larval nematodes were recovered, an incidence of 43% infection. As many as 25 nematodes were taken from one fish.

To the authors' knowledge, this is the first record of parasites of the petrale sole.—Earl Gregoire and Ivan Pratt, *Oregon State College, Corvallis, Oregon.*

[1] Published with the approval of the Oregon State College Monographs Committee. Research Paper No. 182. Dept. Zoology, School of Science.

CIONELLA LUBRICA (MÜLLER), A NEW INTERMEDIATE HOST OF *DICROCOELIUM DENDRITICUM* (RUDOLPHI, 1819) LOOSS, 1899 (TREMATODA: DICROCOELIIDAE)

The lancet fluke, *Dicrocoelium dendriticum,* is a parasite of many species of domestic and wild mammals, which has recently appeared in North America. It is known to occur in the United States only in a restricted area in central New York. Workers in foreign countries have incriminated 14 species of terrestrial snails, belonging to the families Helicidae, Enidae, Chondrinidae, Vitrinidae and Ariophantidae, as intermediate hosts of the parasite. None of these reported species occur in New York State.

To determine the intermediate host here, a malacological survey was made on a farm on which over 200 heavily infected sheep had been pastured for a number of years. The parasitic burden in individual sheep ranged from several hundred to approximately 50,000 flukes. Sixteen species of mollusks, belonging to the families Zonitidae, Endodontidae, Pupillidae, Arionidae, Limacidae, Valloniidae, Cionellidae, Succineidae and Lymnaeidae, were examined in the course of the investigation. The only species found infected with larval *D. dendriticum* was a terrestrial snail, *Cionella lubrica* (Müller).

Although the means by which the definitive hosts become infected was not determined, the following evidence supports the postulation that this mollusk is actually the intermediate host of epizootiologic importance in the area under study. (1) The sporocysts and cercariae found in naturally infected *C. lubrica* were identical with those described by European workers as larval forms of *D. dendriticum.* (2) Trematode larvae recovered from other species of snails in the infective area bore no resemblance to larval or adult *D. dendriticum.* (3) The distribution of *C. lubrica* coincided with known infective areas. (4) Trematode larvae obtained from *C. lubrica* experimentally infected with eggs of *D. dendriticum* were identical with those recovered from naturally infected snails of the same species.

The ubiquity of *C. lubrica* and its holarctic distribution suggest that *D. dendriticum* is destined to become a permanent part of the parasitic fauna of almost all of inhabited North America. Besides being a hazard to the health of domestic herbivores, this parasite is known to affect man.

The writer is indebted to Dr. Henry van der Schalie, University of Michigan, for identifying the mollusks.—Cortland R. Mapes, *New York State Veterinary College, Cornell University, Ithaca, New York.* [This investigation has been supported in part by a Research Fellowship awarded by the Lederle Laboratories, Pearl River, N. Y.]

INCIDENCE OF INFECTION WITH THE FOWL NEMATODE *ASCARIDIA GALLI* IN EGYPTIAN CHICKENS

The intestinal contents of 200 chickens were collected by the flushing method of Ackert and Nolf (1929, Science 70: 310–311). The chickens were secured at random from the group killed at the Kasr El-Eini hospitals during the period from August 26 until December 19, 1950. Infection with one or more species of helminths was found in 172 chickens (86%). One hundred and eleven chickens were found infected with *A. galli.* The ratio between male and female worms in the chickens examined varied from one chicken to another. In a total of 1024 worms

examined for this survey 564 were females and 460 were males. The severity of infection in any chicken varied from one to more than a hundred worms.—S. M. Gaafar, *Veterinary Pathological Laboratory, Giza, Egypt.*

MAMMAL BLOOD PARASITE RECORDS FROM SOUTHWESTERN UNITED STATES AND MEXICO

Previous reports of F. D. Wood (1934, Am. J. Trop. Med. 14, 497–517; 1936, Univ. Calif. Publ. Zool. 41, 133–144), Wood and Wood (1937, J. Parasit. 23, 197–201; 1938, Am. J. Trop. Med. 18, 207–212) and S. F. Wood (1941, Southwestern Medicine, April, 112–114; 1941, Am. J. Hyg., Sec. C. 34, 1–13; 1942, Bull. So. Calif. Acad. Sci. 41, 61–69; 1943, Am. J. Trop. Med. 23, 315–320; 1943, J. Parasit. 29, 363; 1949, Am. J. Trop. Med. 29, 43–55) have recorded blood parasites from mammals of the Southwest. These records with additions reported below are here summarized by states.

With the aid of Denny G. Constantine (78), Loran Whitelock (35), Jim Miles (3), Al Hildinger (3), Ray Johnson (3) and Warren Morton (1), 190 mammals (103 Chiroptera, 69 Rodentia, 16 Carnivora and 2 Lagomorpha) were sampled from California, Arizona, Texas and Mexico. The following infections were discovered: microfilaria in 1 *Sylvilagus bachmani cinerascens* from Griffith Park, Los Angeles Co., and 1 immature *Eptesicus fuscus pallidus* from the Mountaineer Mine, Riverside Mtns., Riverside Co., California; *Babesia* in 1 immature *Procyon lotor psora* from Malibu Canyon, Santa Monica Mtns., Los Angeles Co., California; *Trypanosoma peromysci* in 1 *Peromyscus maniculatus blandus* from Cienega Creek, Pima Co., Arizona; and *Trypanosoma neotomae* in 2 *Neotoma albigula melanura,* and microfilaria in 1 *Neotoma albigula melanura* and 1 *Perognathus penicillatus pricei* from 15 km. N Guaymas, Sonora, Mexico.

During the summer of 1950, blood samples of 215 mammals were studied at the San Joaquin Experimental Range, O'Neals, California and xenodiagnosis was applied to 186 (Wood, 1951, Pan-Pacific Entomologist, in Press). Nathan Cohen, Station Zoologist, supplied 16 blood smears and 14 trapped rodents. Kenneth Stager, Los Angeles County Museum, furnished a "bat kit" for field identification. Twenty-seven infections (12.5%) were found in 92 Chiroptera, 120 Rodentia and 3 Lagomorpha sampled by fresh (199) and stained (215) blood examinations and xenodiagnosis (186). Of these 27 infections, 20 were detected from fresh blood samples or by xenodiagnosis and 7 more were picked up from stained slide examinations. This survey revealed the following infections: *Trypanosoma cruzi* in 2 *Peromyscus truei gilberti; Trypanosoma peromysci* in 3 *Peromyscus truei gilberti; Trypanosoma vespertilionis* in 8 (2 immature individuals) *Antrozous pallidus pacificus* and 1 immature *Eptesicus fuscus bernardinus; Plasmodium* in 1 mature and 4 immature *Antrozous pallidus pacificus;* microfilaria in 1 *Eptesicus fuscus bernardinus; Grahamella* in 4 *Perognathus californicus ochrus; Haemobartonella* in 1 *Perognathus inornatus inornatus;* and *Spirillum* in 2 *Peromyscus truei gilberti.*

In California, 55 or 7.9% of 697 mammals (2 Marsupialia, 268 Chiroptera, 13 Carnivora, 410 Rodentia, 4 Lagomorpha) revealed 39 trypanosome (3 *T. cruzi,* 14 *T. neotomae,* 15 *T. vespertilionis,* 6 *T. peromysci,* 1 *T. microti*), 5 *Plasmodium,* 3 microfilaria, 1 *Babesia,* 1 *Haemobartonella,* 2 *Spirillum,* and 4 *Grahamella* infections. In addition to those infections reported above, *Trypanosoma cruzi* has been found in 1 *Neotoma fuscipes macrotis* from Murray Canyon, San Diego Co.; *T. neotomae* in 12 *Neotoma fuscipes annectens* from Berkeley, Alameda Co., 1 *Neotoma fuscipes macrotis* near La Crescenta, Los Angeles Co., and 1 *Neotoma lepida lepida* from Victorville, San Bernardino Co.; *T. vespertilionis* in 5 *Myotis occultus* from Blythe, Riverside Co., and 1 *Antrozous pallidus pacificus* from 7 mi. W Plymouth, Amador Co.; *T. peromysci* in 1 *Peromyscus californicus insignis* from Simi, Ventura Co., 1 *P. boylii rowleyi* from Lebec, Kern Co., and 1 *P. maniculatus gambelii* from Mill Creek Canyon, San Bernardino Co.; and *T. microti* in 1 *Microtus californicus sanctidiegi* from Murray Canyon, San Diego County.

In Arizona, 14 or 10% of 140 mammals (47 Chiroptera, 4 Carnivora, 3 Primates, 86 Rodentia) revealed 14 trypanosome infections (3 *T. cruzi,* 1 *T. cruzi* with microfilaria, 4 *T. neotomae,* 5 *T. peromysci,* and 1 *T. vespertilionis*). In addition to the *T. peromysci* infection reported above, *Trypanosoma cruzi* has been found in 1 *Neotoma albigula albigula* and 1 *Peromyscus boylii rowleyi* from 7 mi. S Continental, Pima Co., and 1 *P. boylii rowleyi* from 15 mi. NE Nogales, Santa Cruz Co.; *T. cruzi* with microfilaria in 1 *Neotoma albigula albigula* from 15 mi. NE Nogales, Santa Cruz Co.; *T. neotomae* in 1 *Neotoma albigula albigula* from Pinery Canyon, Cochise Co., 1 in same host 30 mi. E Flagstaff, Coconino Co., and 2 at the Alvarado Mine, Congress, Yavapai Co.; *T. peromysci* in 1 *Peromyscus truei truei* from 20 mi. N Flagstaff, *Coconino* Co. and 3 in *P. boylii rowleyi* from Tres de Mayo Mine, 15 mi. NE Nogales, Santa Cruz Co.; and *T. vespertilionis* in 1 *Myotis velifer velifer* from Salome, Yuma County.

Five or 2.7% of 183 Texas Chiroptera revealed 2 *Trypanosoma vespertilionis,* 1 *T. vespertilionis* and microfilaria, and 2 *Plasmodium* infections. *Trypanosoma vespertilionis* was found

in 2 *Pipistrellus hesperus maximus* from the Chisos Mtns.; *T. vespertilionis* and microfilaria in 1 *P. hesperus maximus* from the Chisos Mtns.; and *Plasmodium* in 1 *Antrozous pallidus pallidus* from 10 mi. W Alpine, and 1 *Pipistrellus hesperus maximus* from the Chisos Mtns., all in Brewster County.

Four or 22.2% of 18 Mexican mammals (1 Carnivora, 17 Rodentia) revealed 2 *Trypanosoma neotomae* and 2 microfilaria infections as reported above.

Five Utah rodents and 18 New Mexico mammals (3 Chiroptera, 15 Rodentia) were negative for blood parasites.

Thus, in cooperation with Dr. Fae D. Wood and many collaborating student collectors, the writer here records 78 or 7.3% blood parasite infections from air-dried smears, fresh blood or xenodiagnosis of 1,061 native mammals.

The writer thanks the California Forest and Range Experiment Station and the Division of Zoology at Davis, University of California for use of facilities at O'Neals, California, during the summer of 1950. SHERWIN F. WOOD, *Life Sciences Department, Los Angeles City College, Los Angeles 29, California.*

INTESTINAL PARASITE SURVEYS IN GEORGIA

From November 1946 through July 1948, a total of 926 adults and children in Atlanta, Georgia were examined for intestinal parasites by direct smear and zinc sulphate concentration of a single stool specimen per person. In addition, 145 children were examined only for pinworm infection by cellulose tape slide preparations (Brooke, Donaldson, and Mitchell, 1949, Pub. Hlth. Rep. 64: 897-901). The individuals studied in this survey represented a low-income group of white people whose children were being brought to the Central Presbyterian Children's Clinic in Atlanta for treatment and follow-up of the usual childhood complaints. Normally passed stool specimens were collected from family groups and examined either on the day of passage or on the next day.

Table 1 presents the incidence of the various intestinal parasites found in the group surveyed by stool examination. For comparative purposes, results are presented of a similar survey conducted by Byrd in 1936 (Am. J. Trop. Med., 16: 39-45) in Athens, Georgia. The Athens survey resembles that conducted in Atlanta in three respects: similar techniques were used in both studies; a single, normally passed stool from each person was examined; and the economic levels of both groups examined were about the same. On the other hand, most of the 537 individuals examined in Athens were adults, both white and negro, whereas the Atlanta survey included only white persons, many of whom were children. Except for *Endolimax nana* and *Giardia lamblia*, the infection rates in the two surveys are quite similar. Infections with *Giardia* are known to occur more frequently among children, which probably accounts for the higher incidence of this parasite in the Atlanta survey. The difference in the rates of infection with *Endolimax nana* in the two surveys cannot be explained on the basis of what is known about the epidemiology of this parasite. It should be noted that the percentages given in the table for the protozoa do not represent the "true" infection rates since they are based upon the examination of only a single stool from each person.

TABLE 1.—*Incidence of intestinal parasites in Atlanta, Georgia and in Athens, Georgia as determined by the examination of a single stool specimen from each individual. (Figures in parentheses represent percentages.)*

	Atlanta 1946–1948 926 individuals	Athens 1936 537 individuals
Endamoeba coli	180 (21.6)	139 (25.9)
Giardia lamblia	93 (10.0)	29 (5.4)
Endolimax nana	79 (8.5)	118 (22.0)
Endamoeba histolytica	54 (5.9)	33 (6.1)
Iodamoeba bütschlii	9 (1.0)	8 (1.5)
Chilomastix mesnili	8 (0.9)	4 (0.7)
Trichomonas hominis	7 (0.8)	
Dientamoeba fragilis	2 (0.2)	
Hymenolepis nana	9 (1.0)	3 (0.6)
Hookworm	8 (0.9)	3 (0.6)
Ascaris lumbricoides	3 (0.3)	1 (0.2)
Trichuris trichiura	3 (0.3)	
Strongyloides stercoralis	1 (0.1)	2 (0.4)
Pseudoparasites (Mites, Heterodera)	28 (3.0)	

Of the 145 children examined only for pinworm by a single cellulose tape slide preparation, 34 or 23.4% were found to be positive. Due to the migratory habits of adult female pinworms, the most favorable time to recover the eggs is during the night or immediately upon arising in

the morning. In this survey, examinations had to be made shortly after the noon hour when the children were brought to the clinic. It is likely that a higher incidence of infection with this nematode would have been found if the examinations could have been made at a more favorable time and if additional examinations on subsequent days could have been performed on each person.

The survey reported here is somewhat unique in that it deals with a relatively homogeneous group of people, not institutionalized, who were examined over a period of 20 months. When monthly incidence rates were plotted against the months of the year, no seasonal relationship could be established. Since intestinal parasite infections are believed to be generally long-lived in the absence of treatment, this lack of correlation was not unexpected.

This survey would not have been possible without the excellent cooperation of Mrs. Ralph Nolan, Director, and Dr. T. F. Davenport, Medical Director, of the Children's Clinic.—Morris Goldman and Sadie Johnson, *Communicable Disease Center, Public Health Service, Atlanta, Ga.*

NOTES ON EXCYSTMENT AND CULTURE *IN VITRO* OF THE MICROPHALLID TREMATODE, *GYNAECOTYLA ADUNCA* (LINTON 1905)

Metacercariae of *Gynaecotyla adunca* (Linton 1905) are found encysted in large numbers in the Fiddler Crab, *Uca pugilator*, on Piver's Island, Beaufort, N. C. The green glands of the crab are the most heavily infected organs, although cysts are found also in the digestive gland, gonads, and hemocoele. Merely shaking the green glands in aqueous solutions is often sufficient to free the tissue of most of its cysts. Because of the ready accessibility of the metacercariae and the relatively low degree of host specificity of the adults, these worms were considered excellent experimental material.

The basic aqueous medium for excystment and culture employed with most success is a "1% seawater" solution, obtained by diluting filtered seawater of a known salinity (determined hydrometrically) to a final salinity of 10 parts per thousand (pH 7.4–7.7). After 3–4 washings with 1% seawater, the cysts freed from crab tissue are placed in an excysting solution of the following composition (diluent, 1% seawater):

0.50% Pepsin (Merck, N.F.)	–2.0 ml.
0.038% HCl	–5.0 ml.

Incubation in the excysting solution at 40° C. for 40–45 minutes is followed by washings with 1% seawater until litmus paper shows the cysts to be free of acid. During the incubation period a small percentage of worms excyst, but these usually die in the acid medium. The worms begin to excyst in quantity during the final washing procedure, and an additional 10–15 minutes incubation at 40° C. after these washings will result in 70–90% excystment.

Difficulties in culture arose because of microfloral contamination when organic media were employed. Other workers (Ferguson, 1940, J. Parasit. 26: 359–372; Smyth, 1947, Parasit. 38: 173–181) have reported similar results. For asepsis, penicillin (crystalline G) and streptomycin were tried, and it was found that the latter agent in a concentration of 1:20,000 (Smyth, 1950, J. Parasit. 56: 371–383) was by far the more effective in that it, unlike penicillin, is heat-stable and inhibits the growth of both Gram-positive and Gram-negative organisms. Recent attempts to sterilize metacercariae by repeated washing in sterile salt solutions according to the method of Ferguson (1940; *loc. cit.*) have also met with considerable success. It has been found easier, however, to sterilize the metacercariae by washing before excystment rather than afterward.

Among the culture media tried were extracts and hydrolysates of fiddler crab, fish, bird intestine and other organs, several types of saline solutions in various concentrations and proportions containing yeast extract, Bacto-Peptone ("Difco"), digestive enzymes, bile salts, human and bird whole blood and sera, filtered *Limulus* blood, and autoclaved bacteria. To date, however, the most successful culture medium has been 1% seawater alone with incubation at 40° C. With this medium renewed daily sterility was unnecessary, and worms actively shedding normal eggs were obtained within 80 hours. Sperm can be seen in the ejaculatory duct and cirrus anytime from 1 to 3 hours after excystment, and egg shell formation is evidenced after 10–12 hours incubation.

At this writing, 8 days has been the maximum survival time in culture. In view of the wide variety of culture media tried with little success in prolonging survival time and the rapid maturation of the worms, the authors are considering the possibility that *G. adunca* is inherently short-lived, even in its normal definitive host. Further work is in progress and will be reported in a subsequent communication.

The results described herein were obtained during the summers of 1950 and 1951. The authors are indebted to the Carnegie Research Fund and the Duke University Research Council for financial aid.—Wanda Sanborn Hunter, *Duke University, Durham, N. C.*, and Donald C. Chait, *Medical College of Georgia, Augusta, Georgia.*

OBSERVATIONS ON INFECTIONS IN THE COTTON RAT WITH *LONGISTRIATA ADUNCA* AND *STRONGYLOIDES SIGMODONTIS*

The following observations have been made more or less casually in the course of maintaining a colony of cotton rats for a study of their filarial worms. The rats were housed in wire bottom cages and changed to clean cages monthly. The cages were suspended over trays of sawdust which were changed thrice weekly. All of 20 adult rats caught in Galveston county in the spring of 1950 had natural infections with *Longistriata adunca* Chandler, 1932. The intensity of their infections decreased during the first year as judged by charcoal cultures of the feces. Some infections died out, but most have persisted for at least one year. During the first part of this period nesting material was furnished the mothers on the day before or the day after parturition and not removed until weaning three weeks later. All of 20 litters born to positive mothers during this period became infected. Later the nesting material was removed on the third day after delivery and only 2 of 8 litters were infected. Still later when the intensity of the mothers' infections had decreased, none of 15 litters became infected. The laboratory acquired infections were of light intensity and tended to die out within the first 3 months.

A less careful examination of the wild caught rats was made for *Strongyloides sigmodontis* Melvin and Chandler, 1950, and at least half were found to be infected. Some of these infections have persisted for at least a year under the above described conditions. No infections have been found in litters born to infected mothers.

By segregation it has been possible to establish a section of the colony which remains free of infections with these two species.

A few observations have also been made on the free living stages of *Longistriata adunca* showing that they are essentially identical with those of *L. musculi* described by Schwartz and Alicata (1935, J. Wash. Acad. Sci., 25: 128–146). Newly hatched larvae were about 300 microns in length. Preinfective larvae of increasing size were found in cultures 2 and 3 days old, and ensheathed forms ready to molt measuring about 650 microns were found on the fourth day. Infective larvae were found in various cultures from the third to the sixth day at which time observations were discontinued. Eleven infective larvae measured 617 to 740 microns in length, averaging 682 microns, and 27 to 41 microns in width, averaging 35 microns. The length of the esophagus was from 19 to 27 per cent of the total length. These measurements are all within the limits given for *L. musculi* by Schwartz and Alicata.—J. Allen Scott and Ellen Blynn, *University of Texas School of Medicine, Galveston.*

ON A NEW HOST, BLACK RAT, OF *FASCIOLA HEPATICA*

Fasciola hepatica, a common liver fluke of sheep and cattle, has been reported from a number of species belonging to five orders of the class Mammalia: Marsupialia, Ungulata, Rodentia, Carnivora and Primates. In the order Rodentia, this species has been recorded in members of five families: squirrels in Sciuridae, beavers in Castoridae, coypus in Octodontidae, guinea-pigs in Caviidae, and rabbits and hares in Leporidae. Insofar as we know from literature, no species of the family Muridae in the order Rodentia has been found infected with this trematode.

Recently, in the course of examination of 36 rats in Taipeh, Formosa, I came across two specimens of *F. hepatica* in the liver of a black rat, *Rattus rattus.* The specimens showed all the characteristics of *F. hepatica,* measuring 32 mm. in body length and 13 mm. in width and containing numerous well-developed eggs in the uterus.

Although *F. hepatica* is a common parasite of cattle in Taipeh, its occurrence in the black rat is herewith reported for the first time. This not only adds a new host for this well-known liver fluke, but also indicates the possibility that rats might be used as laboratory animals for the experimental work of *F. hepatica.*

The writer wishes to express her gratitude to Dr. H. F. Hsü, Head of the Department, for his interest in this work.—S. Y. Li, *Department of Zoology, National Taiwan University, Taipeh, Taiwan (Formosa), China.*

THE CERCARICIDAL EFFICACY OF OZONE

Some observations were made on the destruction of schistosome cercariae by ozone, during the time that studies on ozone as a cysticidal agent were under way in this Laboratory. For details of ozone production see Newton and Jones, 1949 (Am. J. Trop. Med., *29* (5): 669–681). Recently shed cercariae of *Schistosoma mansoni* were subjected to concentrations of ozone varying from 0.1 to 1.3 p.p.m. in chlorine-free tap water at temperatures of 27° to 29° C. Action of the ozone was stopped by the addition of appropriate amounts of N/100 sodium thiosulphate. Cercariae were observed for motility under a dissecting microscope. In addition, some of the cercariae were observed under higher magnification for evidence of activity of flame cells.

In the first experiment the applied ozone was 1.1 p.p.m. and residual determinations made at 3, 5, and 10 minutes were 0.9 p.p.m., 0.7 p.p.m., and 0.4 p.p.m., respectively. Cercarial concen-

tration was 50 per ml. Observations for effect on cercariae were made at 1-minute intervals from 1 to 5 minutes and at 10 minutes. No motility was detected after 1-minute exposure, or at any time thereafter.

In two other experiments, cercariae were exposed for only 1 minute to varying concentrations of applied ozone. No residual determinations were made. Cercarial concentration was 2 to 3 per ml. in 100 ml. samples. No cercariae survived exposures to 1.3 and 0.9 p.p.m. ozone. After exposures to 0.7, 0.6, 0.5, and 0.3 p.p.m. ozone cercariae either were killed within the minute or were so affected that they were dead within 30 minutes after treatment. With exposure to 0.2 p.p.m. and 0.1 p.p.m. ozone, cercariae were not killed within 1 minute, but were dead at 30 minutes or 60 minutes post-treatment.

In one supplemental test, cercariae were alive after 2 minutes but killed after 5 minutes exposure to approximately 0.1 p.p.m. applied ozone.

Untreated cercariae in tap water alone or with sodium thiosulphate served as controls and remained active in all experiments.

These limited observations indicate that ozone, in relatively low concentrations, is an effective cercaricide.—M. F. Jones and W. L. Newton, *National Institutes of Health, Bethesda, Maryland.*

BLOOD PARASITES OF SOUTHWEST TEXAS RODENTS

An opportunity to examine a large number of rodents has been presented to us during the course of Q fever studies supported, in part, by a research grant from the Division of Research Grants and Fellowships in the National Institutes of Health. The 2,260 animals taken during the first year of the project (March 1, 1950–February 28, 1951) included over 1,700 rodents, chiefly the pack rat, *Neotoma micropus* and the cotton rat, *Sigmodon hispidus.*

The rodents were trapped alive in Sherman box traps, anesthetized, bled for Q fever serological studies and their ectoparasites removed for laboratory animal inoculation. Inasmuch as it has been demonstrated that rodents are important reservoirs of various blood parasites such as the relapsing fever spirochaetes and the trypanosome responsible for Chagas' disease, thick blood films were made whenever practical. The results are presented here.

Pack rat, *Neotoma micropus.*

Droplets of heart or tail blood from 461 of the animals were stained with Giemsa and examined. Thirty-five per cent or 161 specimens were infected with trypanosomes. Six of the rodents showed spirochaetes similar to relapsing fever *Borrelia.* Thirty-four were infected with microfilariae. Adult filarial worms found in these rodents were determined by Dr. A. C. Chandler to be *Litomosoides.* Six additional rats showed mixed infections of trypanosomes and microfilariae, while 1 rat was infected with both trypanosomes and *Borrelia.* Morphologically, all of the trypanosomes resembled *Trypanosoma lewisi.* Several positive *Neotoma* kept alive in the laboratory retained large numbers of trypanosomes in their circulating blood for months. Attempts to infect laboratory reared *Triatoma lecticularius* and *T. woodi* by feeding them on positive pack rats were invariably unsuccessful. Finally, 15 positive animals were sacrificed and cardiac tissue sections made. No evidence of the leishmania forms of *Trypanosoma cruzi* was obtained. However, it appears likely that the trypanosomes in the pack rat blood represented a mixed infection of *T. lewisi* and *T. cruzi,* since the dejecta of 28 per cent of approximately 100 *Triatoma* taken from the pack rat nests contained *T. cruzi.*

Cotton rat, *Sigmodon hispidus.*

Thick films were made on 400 of these rats and 42 were infected with *Borrelia,* 96 showed microfilariae, 10 additional specimens showed both *Borrelia* and microfilariae, 1 was infected with trypanosomes, and 1 had both trypanosomes and *Borrelia.* The only adult filarial worm found in the cotton rats was *Litomosoides carinii.* During this study, the principal soft shelled tick found in the pack and cotton rat nests was *Ornithodoros talaje.* Whenever a pool of 25 or more of these ticks was fed on a laboratory rat or mouse, almost invariably the animal would become infected with *Borrelia,* indicating that at least one of the ticks was positive. However, it is of interest to note that none of the 10–20 Texas cases of relapsing fever which are demonstrated every year by this laboratory have ever been definitely associated with pack or cotton rat nest *Ornithodoros* ticks.

Pocket mouse, *Perognathus hispidus.*

Eight individuals were examined. Seven were negative and 1 showed *Borrelia*-like spirochaetes.

Deer mouse, *Peromyscus leucopus.*

Of 23 mice examined, 4 showed spirochaetes and 2 microfilariae.

Ground squirrel, *Citellus mexicanus.*

Fourteen were examined. One animal was infected with spirochaetes and 1 with microfilariae.

No blood parasites were found in the limited number of thick films made on the grasshopper mouse, *Onychomys leucogaster* and the kangaroo rat, *Dipodomys ordii*.—R. B. Eads and B. G. Hightower, *State Department of Health, Austin, Texas.*

HEXAMITA (PROTOZOA: MASTIGOPHORA) FROM THE GOLDEN PHEASANT

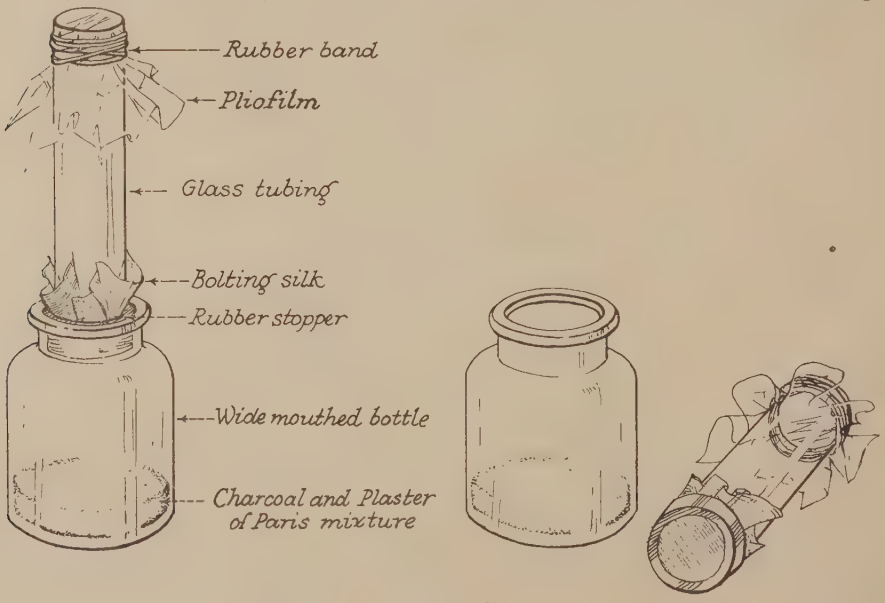
In November, 1950, several golden pheasants (*Chrysolophus pictus*) were submitted by an Illinois zoo to the Diagnostic Laboratory of the University of Illinois, College of Veterinary Medicine. They had been purchased from a professional pheasant breeder about ten days previously. A number of birds had died before the services of the diagnostic laboratory were requested.

The birds were a few weeks old. Only one of them arrived alive and in fit condition for autopsy examination. It was very thin, depressed and unable to stand. At autopsy, numerous *Ascaridia* and one *Capillaria* were found in the small intestine. The ceca were distended, and were filled with a foamy, greyish-yellow, rather thick fluid. *Trichomonas* abounded in this fluid. *Heterakis* was also present in the ceca. Numerous protozoa of the genus *Hexamita* were found in the duodenum.

Hexamita meleagridis McNeil, Hinshaw and Kofoid, 1941 (Am. J. Hyg. 34: C: 71–82) is an important cause of disease in turkeys (cf. Hinshaw, 1948, pp. 1086–1091 in H. E. Biester and L. H. Schwarte, *Diseases of Poultry*, Ia. St. Col. Press). It has been found in the California valley quail (*Lophortyx californica vallicola*), Gambel's quail (*L. g. gambeli*) and the chukar partridge (*Alectoris graeca chukar*) by McNeil, Platt and Hinshaw (1939, Cornell Vet. 29: 331–334), and in the ring-neck pheasant (*Phasianus colchicus*) by Hinshaw and McNeil (1942, J. Am. Vet. Med. Assoc. 101: 503) and by Stover (1943, J. Am. Vet. Med. Assoc. 103: 37). *Hexamita* has also been found in the peafowl (*Pavo cristatus*) (1945, Calif. State Dept. Agric., An. Rept. Petaluma Poult. Path. Lab., Calif. State Dept. Agric. Mon. Bull. 30: 455). *Hexamita* has been transmitted from the turkey to the chicken, quail and duck, and from the ring-neck pheasant, quail and chukar partridge to the turkey. The form found by us in the golden pheasant is morphologically indistinguishable from *Hexamita meleagridis*. In view of the broad host spectrum of this species, it is likely that the golden pheasant form is also *H. meleagridis*.—Norman D. Levine, Paul D. Beamer and Ethel McNeil, *College of Veterinary Medicine and Agricultural Experiment Station, University of Illinois, Urbana, Illinois.*

A HUMIDITY CHAMBER FOR MAINTAINING THE TROPICAL RAT MITE, *BDELLONYSSUS BACOTI*

To maintain tropical rat mites from the time they are fed on cotton rats infected with filarial worms, *Litomosoides carinii*, until the larvae of these worms have reached the infective stage



requires careful control of temperature and humidity. The apparatus described here when kept in a cabinet with temperature thermostatically controlled between 22 and 26° C., provides the necessary high humidity without the formation of dew. As shown in figure 1 the base consists of a wide mouthed bottle containing a small amount of water, or for easier handling a moist layer of plaster of Paris and powdered charcoal. The mites are placed in a pyrex glass tube 22 mm. in diameter and 100 mm. long. The bottom of this tube is covered with 12 XX bolting silk retained by a rubber band over which a ring cut from thick walled gum rubber tubing is slipped to form a stopper. The mites can be sucked into this tube by applying suction through the silk and fitting the top with a rubber stopper pierced by a glass tube with a narrow tip. The mites are then shaken down and the stopper quickly replaced by a sheet of pliofilm held by a rubber band.—J. ALLEN SCOTT, *University of Texas Medical Branch, Galveston, Texas.*

Dates for mailing of numbers of volume 35 (1949):

No. 1, March 21.
No. 2, May 11.
No. 3, July 19.
No. 4, August 5.
No. 5, September 30.
No. 6, December 14.

Dates for mailing of numbers of volume 36 (1950):

No. 1, February 20.
No. 2, April 12.
No. 3, June 2.
No. 4, July 28.
No. 5, November 6.
No. 6, December 16.

Dates for mailing of numbers of volume 37 (1951):

No. 1, February 19.
No. 2, April 11.
No. 3, June 18.
No. 4, August 2.
No. 5, October 29.
No. 6, December 31.

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On recommendation of Council, the American Society of Parasitologists at the annual business meeting, held November 17th, 1951, voted to meet with the American Institute of Biological Sciences at Cornell University in Ithaca, New York on September 8th, 9th and 10th, 1952.

BENJAMIN SCHWARTZ